1971

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B.A. Yale College

1971

Thesis Presented to the faculty of the

Yale University School of Medicine

in partial fulfillment of the requirements for the degree of

Doctor of Medicine

Department of Radiology

Yale University School of Medicine

ACKNOWLEDGMENTS

I own a great debt of gratitude to all persons who have helped me along the course leading to the completion of this study with all its vicissitudes. Among these persons are:

Dr. Gerald S. Freedman - my thesis advisor,

Miss Murita Joell - who worked with me as my assistant during a large part of the study,

Dr. Richard Spencer - who made available the equipment used for the study,

Dr. Robert Lange - who taught me how to use the equipment used in the study and provided many suggestions,

Mr. Joseph Sarmany - who taught me the techniques of handling the rabbits,

Drs. Walker Smith and Arthur Pelligrini - who reviewed the histologic slides with me,

Many members of the Department of Radiology, Division of Animal Care and Histopathology laboratory supplied me with much advice and occasional equipment.

Dr. Morton M. Kligerman has followed my course through the Medical School from initial application to final approaching graduation. He has often found time out from his busy schedule to see me and give advice and admonishment, all in a friendly and concerned spirit. I owe him special thanks.

INTRODUCTION

Radiation therapy is widely used for the treatment and palliation of neoplasms of the lung, both primary bronchogenic carcinoma and tumors metastatic from other primary sites. The limiting factor in the treatment of most tumors is the injury to the normal tissues surrounding the tumor. Prominent among these tissues which limit the dose which can be administered is the spinal cord, but the radiation tolerance of normal lung tissue also contributes to this limit. This becomes of increasing importance in the extended field treatment techniques used in radical therapy for cure. The most sensitive structure in the normal lung, the first to show damage after irradiation, is the vascular system, followed in order of radiation reaction by the alveolar lining cells. Previous studies (Teates, 1968; Johnson, 1970) have shown that one month or more following irradiation there is a decrease in perfusion of irradiated lung tissue which seems to be dose dependent. In both of these reports a review of the data gives indication of an early rise in perfusion following irradiation. This increase is present also in an unpublished study by Kallman. Such an increase could be of importance in the fractionation of radiotherapy since increased oxygenation increases the susceptibility of the cells to radiation damage. The recent availability of equipment which permits easy quantification of blood flow as determined by the distribution of lung scanning isotope has made it feasible to carry out frequent serial determinations of blood flow. Therefore, studies were undertaken in an experimental animal, the rabbit, to determine if any blood flow changes could be observed during the acute phase of radiation injury. It was also investigated whether changes in perfusion had any prognostic significance for the survival of the animal or the subsequent development of radiation pneumonitis or

fibrosis. The extent of radiation injury was also determined histologically at intervals following irradiation. Roentgenographic studies of the development of radiation pneumonitis and fibrosis were not feasible at the time of the present study.

HISTORIC REVIEW

Anatomic Studies

The science of radiology came into being in 1895 with the publication of Wilhelm Röntgen's findings "Ueber eine neue Art von Strahlen" ("On a new Type of Rays.") Soon thereafter it was reported that the new rays were capable of producing skin changes, although this was disputed for some time. One of those who doubted was Prof. Elihu Thomson, who in an effort to find out experimented on himself. He exposed his little finger to X-irradiation for 30 minutes and then observed the severe inflammatory changes which developed over several months. In his report of this experiment he declares himself a firm believer in the ability of X-rays to cause lesions. (1896) Most of the very early papers deal with skin lesions, since the radiation produced in those early days was not strong enough to deliver damaging doses to internal organs. The first tentative reports on X-ray injury to lung and pleura were made by Bergonié and Tessier (1898) who were investigating the possible salutory effects of X-rays on experimental tuberculosis in guinea pigs. They write: ("Does this mean that the invisible radiations have not exercised any effects on the experimental animals? .... But what are we to think of this extended pleural sclerosis, this total symphysis of the inoculated pleura, existing only in the exposed animals, sclerosis similar to that observed by Destot at the level of the cutaneous surface in guinea pigs exposed to radiation ... rapidly striking the whole

extent of the pleural surface, retracting the lung and determining the elevation and flattening of the thorax and diaphragm. ... We will content ourselves for the moment to make known these facts without seeking to know if we should attribute them exclusively to X-rays or if it is necessary to accord a part of the action to the electrostatic field. ... The experimental pathologic results impose cautions and speak, at the moment, against the application of X-rays to human tuberculosis.") (Appendix 1.)

The first irradiation of normal lungs was undertaken in 1909 by Wohlauer. In studies on ten guinea pigs which received high doses of radiation and survived from two days to 2-1/2 months he found hemorrhages, hyperemia and widening of the perivascular and peribronchial lymphatics. The alveolar cells were unchanged, however, and he concluded that these cells were resistant to X-irradiation.

The advent of new and better techniques for irradiation around 1920 made possible "deep X-ray therapy" which resulted in new and more severe radiation injuries. A number of different authors in America and Europe reported clinical signs of lung lesions with the appearance on roentgenograms of fibrosis in those lungs exposed in high doses of radiation. (Groover, Christie and Merrit, 1922, 1923; Case, 1922; Hines, 1922; Wintz, 1922; among others). Hines reported autopsy results of two cases and describes replacement of alveoli by fibrous tissue. Wintz reports induration following radiation of the thorax and breast cancer but describes it as "comparatively harmless" and disappearing in 1/2 to 3/4 years.

These clinical reports of radiation injury and fibrosis following treatment with deep X-ray therapy led to renewed efforts at animal experimentation. Warren and Whipple (1922) studied the differential

sensitivities to radiation of various organs and found that the abdominal organs, especially the small intestinal mucosa, the spleen, the ovaries and the lymph nodes showed reactions at dose levels which produced no demonstrable changes in the lungs. The total dose to the lung was broken up into several small doses to various areas of the lung, however, and thus was not directly comparable to the abdominal irradiation.

Davis (1924) was impressed with the clinical findings in patients who had undergone radiation therapy at the Mayo Clinic and investigated the influence of radiation on the lungs of dogs and rabbits. (Dosages are not given, only radiation specifications.) The microscopic findings in his study were: a) variable degrees of thickening of the alveolar septa, in one case leading to the obliteration of an entire lobe; b) narrowing of the blood vessels in some cases, apparently resulting from proliferation of the media but sparing the intima; c) a definite increase in connective tissue around the bronchi and, especially, the blood vessels; d) in the early stages, a marked edema, filling the airspaces with coagulum and a few desquamated epithelial cells, associated with marked vascular congestion, alveolar capillaries being packed with red blood cells with an excess of leukocytes.

In the ensuing years a number of studies was published. Lüdin and Werthemann (1930) made findings similar to those of Davis but also described degeneration of the alveolar epithelium, a vacuolar reaction of the protoplasm. They also reported proliferative regeneration phenomena of epithelium which appeared to show signs of independent growth, such as polymorphous cells and atypical mitoses, leading to the appearance of a carcinoma-like picture.

Engelstad in 1934 published a major study in which he tried to take

a more systematic approach to radiation injuries than those applied to date, varying dosages and survival times. He described four phases of the reaction of the lung to radiation injury in the rabbit:

> Stage 1. The initial stage, commencing one to two hours after the irradiation and lasting one to two days. Here we find degeneration of the lymph follicles, changes in the bronchial epithelium with abundant mucus secretion, hyperemia, increased transudation and moderate leukocytic infiltration.
> Stage 2. The latent stage, with a duration of two or three weeks.
> Stage 3. The main reaction, with marked degenerative and inflammatory changes. The inflammation may be more or less acute, after very large doses often fulminant. The acute inflammation has its maximum one or two months after the irradiation. Subsequently the leukocytic infiltration slowly diminishes and we then find large quantities of macrophages and often a great number of giant cells.
> Stage 4. A stage with principally regenerative changes, with proliferation of connective tissue and sclerosis, bone production is not uncommon, and there is a slight proliferation of the bronchial epithelium.
> (English translation from Engelstad 1940, p. 676.)

Regarding vascular injury he writes: ("The vascular changes are, as a rule, not pronounced. The capillaries have retained their ability to contract /Adrenalin and Pituitrin/ and their ability to dilate Histamine . For these reasons it appears improbable that the reaction should be ascribed to a primary vascular injury.") (Appendix 2.)

Several important studies emerged in 1940. Warren and Spencer reviewed the histologic findings of 398 autopsies of patients with tumor between the diaphragm and the neck. Using 7 histologic criteria only, they selected 31 cases as showing evidence of prior irradiation, without having any knowledge of the clinical course. Using this method they found only 3 "false positives." Dosages ranged from 600r to 13000r. Their 7 criteria were: 1) a previously undescribed "hyaline membrane" lining the alveolar wall; 2) swollen alveolar lining cells with or without fibrosis, edema or mild inflammatory exudates in the stroma;

3) edema without inflammatory reaction; 4) diffuse alveolar fibrosis without obvious other cause; 5) an unusual fibrillar hyalinization of alveolar wall; 6) hyalinization of arterial walls; 7) interalveolar capillary changes such as swelling of the endothelium and thrombosis. Acute, late and combined types were distinguished. This established that there was a definite, almost pathognomonic. combination of circumstances which were produced by radiation injury. Among their case histories are several cases where the roentgenographic and clinical appearance of radiation fibrosis had been mistaken for spread of recurrence of tumor and led to further irradiation of the area.

Fried and Goldberg (1940) report on a clinical series of 18 patients of whom 11 died and 8 came to autopsy. Their histologic findings support and confirm the findings of animal research. In addition, they noted that right heart failure - cor pulmonale - occurred in several patients and was a frequent cause of death.

By approximately 1940 a great number of reports had been published on radiation injuries to lung, clinical as well as experimental (many of which have been cited here) causing Warren and Gates to comment: "These descriptions are almost monotonous in their uniformity, which contrasts strikingly with the great variations in species used, the conditions of irradiation and the duration of life afterward." Warren in his review article of 1942 pointed out that some of the early reports had failed to distinguish between the effects of radiation and infection, thus leading to erroneous reports of histologic changes. He finds a consensus among reports on most points of the reaction to irradiation: early changes consist of dilatation and engorgement of capillaries appearing within hours of the irradiation, varying in intensity with

the dose. The duration of the reaction also depends on the dose. With heavy doses there is a slight cellular infiltration early in the response occurring as early as 4 hours after a single dose of 1700 rads. There is a coexistence of atelectasis and emphysema, often in adjacent alveoli, which is typical of the radiation reaction. The first cellular changes occur in the respiratory epithelium of the bronchial tree consisting of mucus production and desquamation. Following a latent period a more severe form of cellular injury becomes apparent. The columnar epithelium may show increased mucus production or squamous changes with keratinization and calcification. Anaplastic cells, very large with syncytial character and sometimes showing atypical mitoses may occur weeks or months after heavy doses. In the alveoli there is an increased cellularity "sufficient to cause marked congestion and edema." This is attributed to the proliferation of septal cells. These changes are followed by a filling of the alveoli with alveolar macrophages, their number varying with the intensity of the radiation. They are most prominent 2-3 months after the irradiation. With very heavy doses they have an atypical appearance, forming giant cells and with prominent degeneration and calcification. The epithelial lining cells also react after moderate to large doses of irradiation with swelling, exfoliation, vacuolization and degeneration followed by regeneration. Consolidation occurs, usually in association with bronchopneumonia which in turn is due to a number of factors, such as lowered resistance, focal atelectasis and loss of ciliary action. The severity of the pneumonia varies greatly, causing death in some animals after doses that are well tolerated by others. Distinctive features are only the anaplasia and the hyaline membrane of Warren and Spencer which

occurs only in man and is very nearly pathognomonic. Blood vessel changes occur with edema of the walls of arteries and veins, even after fairly slight doses of radiation. Endothelial swelling is seen after moderate to large doses in the medium and large vessels. Proliferation of the endothelial cells has not been described. After heavy doses, intimal and medial degeneration was seen several months after irradiation. Medial fibrosis occurred with peribronchial and perivascular fibrosis. This thickening of the wall may be due to swelling of collagen in the wall. (Warren, 1942)

Following this large number of reports from 1920 to 1940 not much was written on the subject of experimental radiation injury to the lung. Clinical studies appeared on methods of decreasing fibrosis by use of cortisone or modification of radiation delivery techniques. In 1961 Jennings and Arden published a report on experimental radiation pneumonitis. Their main new findings were that their rats developed a severe radiation oesophagitis leading to a mortality of 80% after 3000 rads and that lung changes were not dependent on the fractionation schedule, only on the total dose delivered. Otherwise their findings were in agreement with the findings of Engelstad (1940) except that they found no latent period in the response. They did not find the hyaline membranes reported by Warren and Spencer (1940).

With the advent of electron microscopy more refined observations became possible. Very few ultrastructural studies in radiation injury occur in the English literature but there are several in German of which that of Bässler and Buchwald (1966) will be cited here. These investigators studied irradiated rat lungs with both light and electron microscopy. The light microscopic studies were in essential agreement with the

results of previous investigators but with a major shift in emphasis. ("The unifying principle for the observed changes could all be referred back to a disturbed permeability of the capillaries, arteries and veins of the lung.") (Appendix 3a.) The vascular changes are thus given a primacy in the explanation of the observed changes. ("Hyperemia and oedema are the earliest and also the characteristic vascular reactions. From the first hours after the irradiation, these forms of irritation are evident and take the form of a widening of the capillaries with aneurysmal ectasis. ... Already after 2 days we observed a perivenous oedema with a loosening of the texture of the wall and swelling of the endothelial cells." /p. 193/) (Appendix 3b.)

> ("The electron microscopic investigations of the radiopathology of the lungs have shown that the alveolar capillary membrane of the lungs exhibits changes within the first 12 hours after unilateral irradiation with 2000 rads, which are partly noticeable after 4 and 8 hours as an increase in membrane and the alveolar lining cells. During the course of the first days after the irradiation there is a rapid increase in the fluid transport mechanism, known as "cytopempsis", which transports fluids from the plasma of the blood, through the endothelium, basement membrane and alveolar lining cells into the alveolus of the lung. The changes in the fine structure show that the oedema fluid reaches the layers of the alveolar capillary membrane transcellularly. In the endothelium collections of watery fluid can lead to extensive vesicular formations. ... With the development of the interstitial inflammation after the second week it becomes clear that the oedema contains more and more protein ... i.e. besides the quantitative increase in vascular permeability there now develops a qualitative effect of a proteinaceous exudate which later also contains fibrin." /p. 202/) (Appendix 3c.)

They go on to say that there is no doubt that the oedema fluid is removed through capillaries, which is indicated by the ectasia of the numerous lymphatics of the involved lung. The above exudative phase is followed by a cellular phase with appearance of neutrophilic and eosinophilic granulocytes beginning a few days after irradiation and lasting for 3-4 months, being replaced by a progressive fibrosis.

The study by Phillips (1966) is not as detailed but also focuses on vascular changes. He notes that beginning on the first day after irradiation there was a segmental separation of the endothelim from the basement membrane in a few capillaries and scattered vacuoles or blebs in the endothelium. (Cf. Bässler and Buchwald above.) These early changes decrease in 4-6 weeks. Beginning at 2 months from the time the study was initiated, the endothelial changes became frequent with widespread vacuole formation and endothelial sloughing. Associated with this was apparent obstruction of the capillary spaces by separated endothelium. During the period 3-6 months after irradiation a cellular reaction was occurring with complete loss of endothelium of alveolar capillaries. Plasma cells appeared in the interstitium. Mast cells appeared associated with collagen, obliterating the previous capillary space. Buds of new capillaries grew into the previous capillary space, developed a new lumen with a new separate basement membrane. These new vessels enlarged to fill the previous space, leaving a thickened basement membrane. The new vessels resembled connective tissue type vessels. Not all new vessels were recanalized. "In general, however, at the 2000 rad level the changes were limited to the endothelial lining and its replacement." These findings are at great variance with many early studies, especially those of Engelstad who could not find occluded capillaries in most rabbits even after careful search. A few individual rabbits formed marked exceptions to this rule.

A current description of the radiation injury reaction of the human lung is found in Spencer's definitive work "Pathology of the lung." (1968):

> Microscopically, the alveolar capillaries are initially the most severely damaged structures and within 24 hours of irradiation they are congested and the alveoli are filled with oedema fluid and macrophage cells. Later, branches

of the pulmonary artery often become thrombosed. Hyaline membrane formation accompanies these changes and the pulmonary lymphatics become very dilated. Later the alveolar lining cells become visible and are greatly swollen, containing enlarged nuclei in which the chromatin is clumped. Some of the cells are multi-nucleated whilst others are attached by narrow stalks to the alveolar basement membrane. Accompanying these changes there is a general polymorph leucocytic infiltration of the alveolar wall, and an accumulation of these cells together with desquamated alveolar epithelium in the alveolar lumen. The shed epithelial cells evince no phagocytic activity. Other alveolar wall changes include fragmentation of the elastic fibres.

By about the fifth day evidence of bronchial and bronchiolar damage appears; at first it is marked by an increased secretion of mucus which is responsible for the troublesome cough, later many of the ciliated cells are shed. The extent of the epithelial damage depends greatly on the amount of radiation received. With massive doses the epithelium is totally destroyed together with the other components of the bronchial and bronchiolar walls, including the elastic tissue. Owing to the loss of ciliated epithelium and excessive mucus secretion, secondary bacterial infection resulting in pneumonia is very liable to occur. In the early stages the pleura, in common with all serous membranes, suffers considerable damage following heavy irradiation. The serosal cells at first become vacuolated and later are shed, resulting in a fibrinous pleurisy; the underlying elastic tissue is also disrupted and the pleural sac may fill with blood and fluid in the early stages.

In the later stages repair results in very extensive interstitial fibrosis of the alveolar walls which become lined by flattened or cubical epithelium. The elastic tissue does not regenerate and the surviving fibres become frayed, thickened and clumped into coarse masses. (Spencer, 1968, pp. 473-475).

Studies on Lung Function

The early reports of disturbances in lung function after irradiation were mostly clinical. Patients having received heavy doses of radiation to the chest were found to develop dyspnea. The earliest reports of this were from Jessen and Rzewuski (1909-10) who made the observation that during X-ray therapy of pulmonary tuberculosis a number of patients developed increased coughing, vomiting and fever. These symptoms later receded. A radiation reaction was considered as cause for these symptoms

but the authors rejected this notion since the phenomenon seemed more consistent with a tuberculous reaction. Groover and his colleagues were the first to point out the connection between irradiation and respiratory symptoms in 1922. Most of the subsequent reports break down into three main groups, but with many mixed reports: Clinical studies of patients with intrathoracic neoplasms receiving radiation treatment; clinical studies of patients with carcinoma of the breast receiving incidental irradiation to the chest with healthy lungs; and experimental studies on animals. Representative studies from each group will be discussed. Physiologic studies dealing with perfusion will be discussed separately. In the first group radiation effects are often difficult to differentiate from those of pre-existing pulmonary disease and intrathoracic neoplasia.

Stone et al. (1956) report on five cases of fatal pulmonary insufficiency following extensive radiation for intrathoracic tumor. Two patients had detailed pulmonary function tests, the remainder only blood gas determinations. All patients received bilateral irradiation to the thorax. The two with full tests showed marked reduction of vital and total capacities in both patients and reduction of residual air in only one. The same two patients had ventilation and gas-exchange studies consistent with alveolar capillary block. The authors relate the amount of insufficiency to total volume treated, total dose, and the rapidity of delivering the dose and possibly cumulative effects from previous irradiation.

Hellman et al. (1964) found radiographic evidence of fibrosis in 100% of patients followed for 30 months after radical radiation therapy for carcinoma of the lung. The doses delivered were 5500 to 6000 rads

in 5-1/2 to 6 weeks. Only five of these had clinical signs of pulmonary radiation reaction. Four of these had clinically evident infection prior to therapy.

Brady et al. (1965) treating patients with carcinoma of the lung tested vital capacity, residual volume, maximum breathing capacity, maximum expiratory flow rate 1/3, specific pulmonary compliance, minute volume, single breath nitrogen test for distribution, arterial pO_2, and found trends in changes but none of statistical significance. Significant changes occurred in arterial pCO_2 and breath holding diffusion capacity. This latter measurement correlated very well with volume dosage of radiation to the thorax. They attribute some of the lack of significance to the fact that most patients have been neavy smokers and showed abnormal results prior to the study, indicating pre-existing lung disease.

Germon and Brady (1968) studied 30 patients all with evidence of pre-existing lung disease and bronchogenic tumors. The pulmonary function tests were essentially the same as in their previous paper (see above). They also performed lung scan on some of the patients. Again none of the parameters of lung function used showed statistically significant changes before and after therapy. There was no correlation with volume dose. The scintigrams, however, showed unexpectedly that even small tumors were capable of producing larger peripheral perfusion defects. This was attributed to either invasion of compression by the tumor of the artery and showed no improvement with radiation therapy. They attributed the lack of correlation between chance in physiological parameters and volume dose in part to the large perfusion defects observed.

Boushy et al. (1970) correlated findings of pulmonary function tests, roentgenographic findings and pathologic findings in a series of patients undergoing radiation therapy for carcinoma of the lung. They found an increase in maximum voluntary ventilation (MVV), a decrease in expiratory resistance, and a decrease in the diffusion capacity for CO (DLCO). The remainder of the pulmonary function tests were not significantly changed. Patients with chronic obstructive pulmonary disease showed more improvement in ventilation than those who were normal prior to therapy. The change in DLCO was not related to radiographic or pathological changes in the lung parenchyma or to radiation dose. The authors speculate that it could be due to ventilation-perfusion imbalance, either from changes in perfusion, such as reported by Johnson et al., 1968, (see below) or ventilation disturbances from fibrosis in the airways. The study found great variability in responses to radiation but found good correlation between roentgenographic and pathologic means for detecting radiation changes.

In the second group of reports a number of studies have been published. McIntosh and Spitz (1939) in treating carcinoma of the breast correlated symptoms with the severity of the observed radiographic changes after radiation. In their radiographic groups III and IV (large and confluent areas of exudation, retraction of associated structures with contracted lung) they found 10 of 15 cases with symptoms of cough for 4-6 months, bronchopneumonia in 5 patients. There was apparently no dyspnea. Symptoms showed no correlation with dose, with the patient getting the largest dose being without symptoms.

Leach et al. (1942) found cough, decreased vital capacities and dyspnea in 12 patients who had undergone treatment for carcinoma of the

breast. The severity of the cough was directly related to the amount of dyspnea. They attribute the decrease vital capacity to " a) fibrosis of the lung /diminishing the volume/, b) a variable degree of fixation of the chest wall, 3) compensatory emphysema in the undiseased lung, d) hyperirritability of the Hering-Breuer reflexes in the fibrotic pulmonary tissue." (p. 745)

Bate and Guttman (1957) treated 50 patients with carcinoma of the breast. Thirty-five showed radiographic changes. Seven of these developed symptoms of whom only three had dyspnea. All the symptomatic patients were in the group of 20 patients given 5000 rads or more to the chest wall.

Gish et al. (1959) in a study which primarily explores the time-dose relationship and the development of radiation reactions also tested 24 patients for changes in total and 3-second vital capacities. Radiation doses ranged from 2000 rads in 5 days to 8200 rads in 30 days. A reduction of 10% or more was found for vital capacity in 9 cases (38%) and 3-second vital capacity in 11 cases (46%). Maximum changes were 18% and 22% respectively. No patient showed clinical signs of impaired pulmonary function. Interestingly, they found that the 10% or greater drop in vital capacity seemed to correlate better with the presence of disease as indicated by the subsequent finding of metastaes, than with the reports of fibrosis roentgenographically. (The report above by Germon and Brady /1968/ of perfusion defects associated with tumors may help to explain this finding - through reflex alterations in ventilation perfusion ratios in the ischemic lobes.)

Cooper et al. (1961) studied a mixed group of breast and intra-thoracic neoplasms using physiologic evaluations: timed vital capacity, maximum breathing capacity, and arterial oxygen percentage saturation.

Studies on lung cancer patients were made difficult by the rapid and high incidence of recurrence making it impossible to determine if observed effects were due to disease or radiation reaction. When the primary was in the lung the improved function due to reduction in size of the tumor was usually enough to offset decreased function due to radiation reaction until recurrence occurred. "In general, when the lungs were normal before irradiation, the severity of untoward changes, whether measured by physiologic test, clinical symptomatology, physical signs or roentgenographic findings was in proportion to the amount of radiation given to pulmonary tissue." Pre-existing pulmonary disease was aggravated, leading to a diminished tolerance for large doses of radiation. Routine physiologic tests proved to be sensitive indicators of radiation reactions, being more sensitive than clinical symptomatology or chest X-ray. Ventiliatory functions were regularly impaired with acute radiation reaction and progressed in the chronic stages of reaction. Diffusion capacity was decreased during the acute phase only. In a follow-up of the same patients and others, Teates and Cooper (1966) found 5 of these (originally 28 patients) still alive and also reported on 11 others. In this series, however, they found 50% incidence of roentgenographic changes consistent with radiation reaction but significant reductions in ventilatory function in only 25%, all of whom showed X-ray evidence of changes. However, three patients with abnormal diffusion had normal chest films. They go on to discuss the variables in physiologic testing such as patient cooperation and the fact that compensatory changes take place in the lungs so that overall measurements may not reflect the amount of actual functional impairment in the irradiated lung. The conclusion of the second paper, therefore, is that physiologic studies are <u>not</u> as sensitive as

roentgenographic examination in detecting the radiation damage in the lung.

Emirgil and Heinemann (1961) studied extensively 15 patients receiving radiation therapy to the lung. Doses ranged from 3100 rads to 5000 rads given over 5-6 weeks. They employed several different parameters defining ventilation, perfusion capacity and the work of breathing. The first measurable change was a progressive reduction in lung volume, beginning 1-3 weeks after the completion of therapy. Following this there was a hypoxemia with oxygen desaturation and an oxygen tension gradient between alveolar air and arterial oxygen. This gradient appeared before any measurable changes in diffusing capacity and were explained as an increased admixture of venous blood being shunted through poorly ventilated areas of injured tissue. Two to five months after onset of radiation therapy there were transient reductions in the diffusing capacities returning back to normal except in two patients with bilateral radiation fibrosis. This apparently indicates that the return to normal in patients with unilateral fibrosis is on the basis of a functional compensation with reduction in ventilation and perfusion of the affected area. A progressive increase in the work of breathing was noted, consisting of decreased maximal breathing capacity due to decrease in lung compliance rather than an increase in airway flow resistance, which had little or no effect. The decreased lung compliance appeared to be due to several factors, only one of which was the radiation injury to lung tissue. Other factor suspected to be involved were positions of mediastinum and heart and possible effects from secondary infection during the radiation pneumonitis. These authors too believe that physiologic compensation may mask the extent of the original injury but that qualitatively the changes were similar

to those observed.

Experimental Physiologic Studies

Experimental physiologic studies have borne out clinical observations and added more data. Sweany et al. (1959) irradiated the thorax of dogs, giving doses ranging from 1000 rads in a single dose to 4800 rads in fractionated doses. Various parameters of pulmonary function were studied. Total thoracic complicance decreased at 4 weeks with greater decreases at 11 and 19 weeeks. Studies showed no change in thoracic wall compliance with the entire decrease occurring as lung compliance. Diffusing capacity began to show signs of returning towards normal in 2 of 3 dogs at 7 and 9 months following irradiation. Functional residual capacity showed progressive decreases beginning at 12 weeks following irradiation. Pulmonary arterial pressures, pulmonary venous pressure, cardiac output, and pulmonary vascular resistance did not change significantly up to a period of 5 months following irradiation. In 3 animals studied after 6 months pulmonary arterial pressures and resistances seemd to be elevated. Pathologic studies showed only dilated capillaries at 4 days. Focal atelectasis with fibrosis and hyperemia were seen at 4 to 5 months. At 6 months and more there was interstitial fibrosis and paucity of cellular elements and capillaries. The small pulmonary arteries showed marked narrowing due to endothelial proliferation. Radiographs showed no obvious abnormalities during the study period. The discrepancy between observed physiologic changes with onset between 80 days and 6 months and pathologic changes which occur within several days and continue for weeks is attributed to possible physiologic compensations which prevent detectable changes prior to this

time. One possible mechanism would be pulmonary venule constriction occurring in response to stimulation. Capillary dilatation and oedema formation would be expected and diffusing capacity might remain unchanged due to the offsetting change oedema and increase capillary blood volume. However, absence of early changes in compliance argues against this theory since oedema and vascular congestion would decrease compliance.

In a significant paper, Teates (1965) showed changes in pulmonary function in dogs. One hemithorax was irradiated with 4500 rads over 23 to 27 days and changes in physiologic parameters for each lung were measured by means of a differential spirometric technique, using tracheal dividers which permitted the study of each lung separately. The magnitude of the changes in the irradiated areas are somewhat uncertain since the field of irradiation extended only to the highest point of the dome of the diaphragm so that all the lung tissue caudal to a plane through that point was not irradiated. Compensatory changes in this unirradiated portion of the lung under study decreased the magnitude of the changes observed. It appears highly unlikely, however, that any qualitative differences in observed changes would occur due to this, and thus only quantitative data are affected. The parameters studied were minute ventilation, diffusing capacity, O_2 consumption, CO_2 production, and compliance. Of these only diffusing capacity showed any significant change in the first 6 weeks, showing a "highly significant" increase in the irradiated lung in the first three weeks after irradiation. Compliance and inspiratory capacity showed small but "significant" decrease in the irradiated lung during the first week but not during the third week. After 6 weeks all parameters showed sharp decreases in the irradiated lung. There had

been no signs of reversal toward normal at the time of sacrifice of the animals approximately 200 days after radiation. (The radiation was given over a 23-27 day course, "days post irradiation" is not further defined but presumably refers to conclusion of irradiation.) The pathologic examination of the lung showed changes consisting of focal areas of fibrosis and thickened alveolar walls and pleura. Alveolar lining cells were enlarged. Bronchial mucosa and blood vessels showed little change. These changes were classified as the "predominantly regenerative stage" of Engelstad (1934). Because of the similarity in magnitude of the changes in oxygen consumption and CO_2 production it was felt that an alveolar-capillary block was unlikely as cause for the observed changes in diffusing capacity. These parameters reflect pulmonary blood flow, however, the magnitude of the changes in diffusing capacity make necessary of another explanation such as ventilation-perfusion disproportion of decreased alveolar ventilation. The observed early increase in diffusing capacity is not explained by any of the observed concomitant changes.

Changes in Blood Flow

Studies of blood flow have long been hampered by the need for operative interference of some sort in order to obtain detailed information. Arteriography cannot be performed on all patients and can yeild only approximate quantitation of blood flow. Catheter studies are usually prolonged and associated with a certain morbidity and mortality. Spirometric studies are time consuming and even more of a strain on the patient's welfare and will to co-operate. As a result, clinical information on blood flow, and in particular pulmonary blood flow was hard to obtain and consisted mainly of morphologic

evidence of vascularity in radiographs. With the development of lung scintiscanning (to be described in detail below) and its application to clinical problems in 1964 (Wagner et al.) a powerful new tool came into the hands of the clinician and researcher and all recent papers, both clinical and experimental, have used this technique.

In papers published the same year, Johnson et al. (1966) and Germon and Brady (1966) describe similar phenomena. Johnson and co-workers show that the perfusion defect detected by lung scanning in patients with intrathoracic mass lesions often were grossly out of proportion to the size of the lesion. Nine of 25 patients, all with lesions involving the hilus, had areas of ischemia much larger than the lesion, in one case involving ischemia of the entire lung. (Three patients with lesions at the hilus did not show this phenomenon.) These patients were treated with radiation therapy to the thorax and perfusion changes were followed by lung scintiscans. Seven of 16 patients showed enlargement or de novo appearance of areas of ischemia (of the remainder 8 showed no change and one, the patient with total ischemia of the lung, showed improved perfusion.) The perfusion defects corresponded to, or were larger than, areas showing radiation pneumonitis and fibrosis on the chest film.

Germon and Brady made the same observation that centrally located tumors produced large perfusion defects. However, in their series all patients showed decrease in perfusion after radiotherapy. They speculate that impaired perfusion may lead to decreased production of surfactant and ensuing atelectasis.

Goldman et al. (1969) studied perfusion patterns in patients who received thoracic irradiation. Of 36 patients who were studied for at

least 3 months, 12 had evidence of radiation pneumonitis and/or fibrosis. Seven of these showed changes on lung scan and chest radiograph, 2 on scan only, and 3 on radiograph only. On those where changes were detectable by both techniques the areas of involvement were of corresponding size.

Experimental Studies on Blood Flow Changes Following Irradiation

A search of the literature has revealed only 2 experimental studies on the effects of radiation on pulmonary blood flow distribution. Both employed lung scanning procedures. Teates (1968) irradiated 3 dogs with 3000 rads over 5 days to the right lung and followed blood flow serially by means of densitometric quantification of the lung scintigraphs. The dogs were followed also by means of chest radiograms and pulmonary arteriography was performed just prior to sacrifice, approximately 13 months after irradiation. A statistically insignificant increase in blood flow to the irradiated lung occurs in the early post-irradiation period according to Fig. 1 in Teates' paper but this is otherwise not commented on. Roentgenograms showed slight alveolar infiltration beginning about 7 weeks after irradiation, followed by changes in the lung scan. The peak of infiltration occurred at 11 to 15 weeks. Consolidation with loss of volume occurred, clearing by 30 weeks but leaving persistent volume loss. This loss of volume was also clearly evident on arteriography. Pathologic examination showed marked fibrosis, enlarged alveolar lining cells, and marked thickening of the media and intima in some vessels. In discussing the findings, Teates points out that the onset of radiation pneumonitis is detected about as early with lung scans as with roentgenography but the residual perfusion defect after resolution of the

infiltrate is shown only by the scan. The mechanism of the perfusion changes remains unclear, being either a physiologic response to local anoxia or a primary vascular injury. Arteriograms showed the major arteries to be patent in the irradiated lung. Thus the changes apparently take place in vessels smaller than the limit of resolution of arteriograms, approximately 0.5 mm, leading to a reduction of flow through vessels 30-40 microns in diameter, the size of the vessels which normally entrap the lung scanning label. Combinations of these factors may operate.

The other experimental study is by Johnson et al. (1970). Using mice they irradiated one hemithorax with doses ranging from 100 rads to 4000 rads in a single dose. Animals from each group were sacrificed at intervals from 4 days to 10 months. Before sacrifice, lung scanning isotope (^{131}I - MAA) was injected and the amount of radioactivity in each lung was measured after removal of the lung using a well counter. Perfusion per gram of tissue was calculated and expressed as the ratio of the irradiated to the control lung. No pathologic examination was performed. Fig. 4 of the report shows that animals in all dosage groups have perfusion levels which (minimally) exceed control levels at 4 days post irradiation. This increase is not commented on. Reduced perfusion ratios were found in all groups of animals studied beyond 1 month post irradiation. Reduction of flow greater than 2 standard deviations from control level were found at 1 month in the 4000 rad group, and 6 months in the 100 rad group. In most categories of time and dose, the variations within the group are fairly great, the median standard deviation being 0.21 for ratios ranging from 0.45 to 1.09. All the changes observed showed no signs of reversal at the time of

longest survival, 10 months, which is approximately half of the life span for the experimental animal.

Kallman et al. in an as yet unpublished study investigated the influence of radiation on the perfusion of mouse sarcomas using ^{133}Xe dissolved in saline. Most of the studies were carried out on unanesthetized animals since it was discovered during the course of the study that pentobarbital anesthesia influenced blood flow. They found an initial decrease in perfusion occurring at three hours following irradiation in tumors which received 1000 rads or more. The decrease was larger with larger doses. Following this an increased flow was noted, peaking at 3-4 days following 1000 rads, 5 days following 2000 rads and 7-9 days following 4000 rads. These increases apparently reached statistical significance at least on the day of peak flow. The rate of blood flow did not increase in tumors receiving 8000-16000 rads. They state that the increases in blood flow were not correlated simply with gross tumor shrinkage. A speculation is made that this radiation-induced blood flow change is related to the tumor reoxygenation following irradiation described previously.

METHOD

Development of Lung Scanning

The use of isotopes as diagnostic tools had to await the development of a light-weight, directional radiation detector which could be developed into a scanner. (A scanner consists of a highly directional radiation detector which can be passed over the body or an organ in a systematic, linear fashion to produce an image of the location and intensity of radiation sources in the tissue over which it is passed.)

One such detector was described by Cassen et al. (1950), having an aperture of 1/4 inch, giving a theoretical limit of resolution of 1/4 inch. The following year (1951) Cassen et al. described the development of a single track scanner. In an effort to improve the speed and/or resolution of the scanner, Anger (1953) introduced a 10 track scanner. Because of the size of the detector the spacing between the channels was fairly great and in order to obtain a high resolution scan an overlapping scanning pattern was used. Using this method and equipment he presented a case of localization on total body scan of distant metastases from a thyroid carcinoma, using radioiodine as the tracer. Anger (1952) also introduced a prototype for the gamma camera, using a pinhole collimator projecting an image onto a flourescent screen with an apposed X-ray film. The idea employed in this prototype - projecting a gamma ray image on a screen capable of converting the radiation energy into visible light energy which is then recorded by a photosensitive device - was refined and developed into the Pho/Gamma III gamma camera, the equipment used in the present study. It consists of an 11 inch diameter, 1/2 inch thick sodium iodide crystal, producing scintillations when struck by high energy photons from a radiation source. Above the crystal are located 19 photomultiplier tubes which record the scintillation and locate it on a 64 x 64 grid in the display system by comparing the relative intensity of the scintillation event as recorded by each tube. The characteristics of this system have been examined. (Freedman et al. 1969) They found a "halo" effect from the increased response at the edges of the field. Also the resolution of the system is energy dependent, as well as being dependent on the collimator used, reaching a maximum resolution of 1 cm at energies above 280 keV with

a pinhole collimator. In a study on collimators, Hayes (1970) states that "although the pinhole collimator has superior resolution its severe image distortion and relatively poor sensitivity are serious obstacles to its clinical use for lung and liver-spleen scanning." However, these drawbacks are of less importance in studies where resolution is of great importance and the time factor is immaterial (such as in the present study.) The distortion is of great importance when the shape of the organs being scanned is of great importance, but much less so in quantitative studies. Response characteristics can further be corrected in the present system by using a normalized field flood as standard for correction.

In the development of scanning techniques the problem early became one of how to selectively deposit isotopes in the organ under study. Physiologic functions of the various organs formed the basis for isotope accumulation. The iodine concentrating properties of the thyroid had already been utilized. Halpern et al. (1956) used the phagocytic properties of the reticulo-endothelial system to trap radioactive particles and performed liver-spleen scans. Radioactive particles (tagged albumen aggregates) in the bloodstream provided an estimate of the amount and distribution of blood in various tissues. Larger amounts of isotope could be used because the vehicle, albumen, is metabolizable and does not remain in the R-E system like other substances used. Attempts at lung scanning were made using ceramic microspheres containing radioactive mercury isotopes. (Haynie et al. 1963) These had the drawback of exposing the lung to excessive radiation. A breakthrough occurred in 1963 when Taplin et al. reported on the preparation of a macroaggregate of human serum albumen (MAA) which could be labeled with

a suitable isotope and used for lung scanning. Advantages of this method was that it employed a physiologic substance as a vehicle which was readily broken down after a few hours so that the radiation source was no longer bound in the tissue but could be released to be excreted in the urine (or by other routes.) This permitted the administration of much larger doses of isotopes, improving the efficiency and resolution of the scan. Variations on this principle have been introduced (e.g. among others the ^{113m}In bound to gelatin particles used for the acute phase study in this experiment.)

With the introduction of the macroaggregates lung scanning became a clinically feasible procedure. The equipment had already been developed. The early studies focused mainly on major aberrations of blood flow to the lungs such as pulmonary embolism (Wagner et al. 1964) and compromise of blood flow by tumors. (See Johnson et al.; Germon and Brady, above.) Only comparatively few quantitative studies have been done, almost all experimental. Lopez-Mejano (1964) introduced a method of quantifying blood flow by measuring the optic density of the scintigraph obtained on X-ray film. There has apparently not been any application to date for the numerical quantification of blood flow in clinical work. The conventional scintigraph used in clinical practice makes an estimate of blood flow by estimating the density of the film by eye.

Measurement of Blood Flow from Lung Scans - Theory and Validation

Many methods have been devised to measure blood flow. Among these are the dye dilution techniques and the Fick Principle, which measures the amount of a substance which has been added to or removed from circulating blood and the change in concentration of the substance

in the blood. Radioisotopes are also used. In general, techniques of blood flow measurements are based on the principle of conservation of material (Wagner and Tow, 1967, from whom much of the following discussion is derived.) The total amount of a material flowing into an organ or area per unit time, Q, can undergo one of several fates: a portion may leave the area, q_e; a portion may remain in the organ, q_i; and a portion may be metabolized, q_m. According to the principle of conservation of matter we get:

$$Q = q_i + q_m + q_e$$

The amount flowing into an organ per unit time is the product of flow and concentration of the indicator substance. Thus:

$$Q = F \times C \qquad \text{(mg/min = ml/min x mg/ml)}$$

where F is flow per unit time and C is concentration, Substituting for Q:

$$F \times C = q_i + q_m + q_e$$

If the indicator is not metabolized then $q_m = o$, and if the indicator substance does not leave the organ or region then $q_e = 0$ as well. Thus, in such a case

$$F \times C = q_i$$

i.e. for a given concentration the flow into an organ or region is proportional to the rate of accumulation of the indicator substance in that organ or region, under the conditions specified above. The indicator may not be present in a steady flow into the organ or region but only for a moment when delivered in a bolus. In this case, the amount accumulated at the moment of flow through is proportional to the flow at that moment assuming that the bolus is uniformly mixed with the blood. The flow can be estimated by measuring the amount accumulated in the organ or in a region of an organ.

This forms the theoretical background for the estimation of blood flow from lung scans. The macroaggregates of albumen (MAA) are not metabolized to any significant extent during the period of time required for the study. The MAA particles (90% of which have a diameter of 10-90 microns, with a mean of 35 microns) are filtered out by the pulmonary capillary bed, thus fulfilling the conditions of the above equation. In actual studies of the extraction efficiency, Tow and associates (1966) using dogs and rabbits found that 77.2$\pm$ 15.1% of the dose administered from their own preparation of MAA could be shown to have remained in the lung. In similar studies on man they found an extraction efficiency of 80% which according to the report "does not introduce an appreciable error in the estimation of regional pulmonary arterial blood flow." (Tow et al. 1966, p. 666) Of the remaining 20% in the systemic circulation only a very small proportion enters the bronchial arteries and "therefore, the regional flow, as measured by the MAA method is only pulmonary arterial rather than total flow." (p. 666) The particles which enter the systemic circulation are removed by the reticuloendothelial system of the liver and spleen.

Several investigators have addressed themselves to the problem of showing that the distribution of the isotopes into the pulmonary vascular bed actually reflects the distribution of blood flow to these areas. Wagner and Tow (1967) mixed ^{125}I labeled MAA and ^{51}Cr labeled red blood cells in a syringe and sacrificed dogs 7-10 seconds after intravenous injection of this mixture, using electric shock to cause immediate circulatory arrest. Samples of lung tissue were measured in a well-type detector using gamma-ray spectrophotometry to measure ^{125}I and ^{51}Cr. Using this method they found a correlation coefficient

of r = 0.97 between the distribution of MAA and red blood cells given by the same injection.

Lopez-Majano and associates (1964) determined pulmonary blood flow by means of differential bronchospirometry according to the method of Björkman (1934). This was correlated 3-10 days later with a pulmonary scintiphotogram. Optic density of the photographic film in 9 scattered areas over the region of each lung was determined to measure the distribution of the isotopes. Using this method they showed a correlation coefficient of $r = 0.96$ ($p < 0.001$).

The optic density method for determining the distribution of the isotope was criticized by Isawa (1966) who pointed out that in areas of high scan intensity there is overlap of spots and saturation of the density of the film. He also regards the use of selected spots as "arbitrary" and making exact positioning of the film of critical importance. In his own study he correlates blood flow as determined by differential bronchospirometry with the integrated area under a curve traced by a rate meter connected to the rectilinear scanner. Using this he obtained a correlation coefficient of $r = 0.986$ ($p < 0.001$).

Tisi et al. (1967) reject bronchospirometric methods to determine blood flow and densitometric methods to determine distribution of isotopes as "indirect." In their study, electromagnetic flow probes were attached to the main pulmonary artery and a branch pulmonary artery to obtain direct measurements. The distribution of isotopes between the two lungs was determined with a scintillation camera providing digital readouts of the radiation in each lung studied separately. They found low correlations with the lungs studied *in situ* but excellent correlation ($r = 0.8$; $p < 0.001$) when the excised lungs were studied separately.

They attribute this discrepancy to the artefactual chance in intrathoracic conditions caused by the thoracotomy necessary to place the flow probes.

Thus, several different investigators using different methods have found very high correlations between the distribution of blood flow and the distribution of radioisotopes injected into the bloodstream and trapped in the pulmonary capillary bed. The distribution of isotopes can thus be regarded as a true estimate of relative pulmonary blood flow. Absolute values for flow cannot be determined however, without reference to data provided by another method, e.g. Fick principle.

Other experiments have been performed to determine the effects of the method of investigation on the animal employed for the studies. The possibility of effects on hemodynamics being introduced by the process of lung scanning has been investigated. Wagner (1964) found no elevation of pulmonary arterial pressure, respiratory rate, or femoral artery pressure in dogs after 10 mg/kg of MAA were injected, a dose 2000 times greater than the doses commonly used for diagnostic lung scans in man. A lethal dose in rats was more than 5000 times the diagnostic dose in man. Rats who received 10 mg/kg were indistinguishable from controls when examined histologically immediately after injection and 1 week later. In more than 1200 studies with MAA in more than 300 subjects there was no evidence of antigenicity of the human albumen aggregates. Studies were carried out in man, dogs, rabbits, and guinea pigs. When Lugol's solution was used to block the thyroid, 80% of the radioiodine was excreted in the urine in 48 hours, with the entire dose being accounted for over several weeks. The calculated radiation dose to the lung from an injection of 300 Ci of ^{131}I was 0.3 rads.

Gold and McCormack (1966) found no change in any of a comprehensive battery of pulmonary function tests including ventilatory functions and gas exchange functions. They write: "Although emboli lodging in vessels the size of arterioles may produce changes out of proportion to their number by inducing reflex or humoural reactions in the lung a threshold number of particles must reach the lung before the reaction occurs. Our findings, therefore suggest that either the emboli are too few or they are of such a size as to miss reflexogenic areas." Their conclusion was that lung scanning was safe even in the face of pre-existing pulmonary disease.

Tisi et al. (1968, see above) using flow probes found that following injection of an adult human dose into a dog, there was "no change in the mean systemic arterial pressure, pulmonary arterial pressure, or total pulmonary blood flow." Serial analysis of arterial blood oxygen tension (Pa_{O_2}) while the dog was ventilated with 100% O_2 disclosed no change of Pa_{O_2} following injection of MAA.

Tow et al. point out (1966) that there are 2.8×10^{11} capillaries in a human lung but only 2.5×10^5 particles in a mg of protein. Only about $1/10^3$ capillaries are blocked by the lodged MAA particles, following the injection of an ordinary dose as various size particles block capillary and precapillary vessels.

In studying the effects of gravity on the distribution of blood flow to the lungs in rabbits, Fernandez et al. (1969) used pulmonary scintigrams and calculated blood flow according to the method of Tow (1966) (Densitometric studies of lung scintigrams.) Rabbits were strapped to a board which could be rapidly tilted from the horizontal to the vertical plane. Injections were made with the rabbit being

horizontal and at various intervals after having been raised to the vertical position. The findings were that ("in the first 15 seconds which follow the placement of the animal in the orthostatic position, the pulmonary blood mass follows the law of gravity and collects at the bases. On the contrary, when the tracer injection is made 20 seconds, 40 seconds, 1 minute and longer after the animal is placed in the vertical position the distribution of blood becomes homogeneous in the two [upper and lower] lung fields and it is no longer possible to find a significantly different distribution from that which has been observed with the animal in the horizontal position.") (Appendix 4.) They also found that the time from injection to entrapment in the lung of the MAA particles was 3-4 seconds.

Methods and Materials

The animals used in this study were mostly male white rabbits weighing between 2000 and 3000 g. at the outset of the study. (The first shipment of 14 rabbits contained a number of larger animals and a few females.) Three groups of rabbits were used, with random assignment to categories within each group. One group of 25 rabbits was used for serial studies of blood flow changes with long term follow-up after single doses of 750, 1500, and 3000 or 3500 rads. One group of 4 rabbits was studied for acute blood flow changes following very high single doses (4500 - 6000 rads.) A third group of 16 rabbits were sacrificed at intervals from 3 to 60 days post irradiation with doses similar to the first group to obtain histologic correlates of the observed blood flow changes. One rabbit was studied to determine rate of disappearance of MAA from the lung. At the time of study the follow-

ing procedure was followed: The rabbit was restrained sitting up in a rabbit box. The tracer injection - usually ^{131}I-MAA - was injected intravenously into an ear vein before the animal had received any anesthetic or other medication. Scopolamine HBr 0.25 mg was injected. Sedation was achieved with intravenous sodium pentobarbital (Diabutal brand) in varying dosage. (Unirradiated animals received 25 mg/kg, irradiated animals received 10 mg/kg because experience showed that they tolerated only much smaller doses.) In order to achieve complete anesthesia ether was administered by inhalation via an improvised ether mask. At the time of irradiation the procedure for anesthesia was the same with omission of the initial injection of the tracer. All rabbits received Lugol's solution in the drinking water throughout the study to block the thyroid uptake of radioiodine.

Radiation was delivered to the right hemithorax using a collimated beam from a Siemens "Stabilipan" X-ray source. The specifications for the irradiation were: 250 kV, 15 mA, 40 cm target to skin distance, 5 x 10 cm field, filter 2 mm Al. This combination delivered 160 rads per minute skin dose. The anesthetized rabbits were restrained lying supine on a restraining board. The antero-posterior diameter of the thorax was oriented vertically. The field was localized using anatomic landmarks. The medial border of the field was along a line drawn from the xiphoid process to the jugular notch. The inferior border ran through a point on the costochondral arch of the free margin of the rib cage, lying midway between the midline and the midlateral line ("the mid axillary line"). The field thus extended below the highest point of the diaphragm in an attempt to include the posterior and lateral costodiaphragmatic recesses and the lung contained within them.

The lateral margin of the field fell outside the alteral wall of the thorax. The superior margin of the field varied slightly with the size of the rabbit but included the humeroscapular joint and portions of the neck. The apex was included in all cases. (See Fig. 7.)

The studies were carried out with the rabbit anesthetized and restrained as described above. The Pho/Gamma III Anger camera with a pinhole collimator was used. (By using object-to-pinhole distances which were shorter than the pinhole-to-image distances optical magnification of the image was achieved, facilitating the calculations.) The data were stored on magnetic tape and a Polaroid scintiphoto was obtained simultaneously. The calculations were carried out on a Nuclear Data 50/50 computer in the following manner: The digitized image of the lungs was retrieved from the tape storage into the computer and displayed on the screen. The image was corrected by the computer to eliminate "collimator aberrations" inherent in the system using a uniform field flood as a standard for correction. A rectangular "frame" or area of emphasis was placed around the image of the right lung so that it approximated as closely as possible the area occupied by the lung. The medial border of the frame was the most critical aspect of this operation. Care was taken to place the frame so that it divided the hilar area in half, including approximately the same amount of hilus with each lung. After the frame had been placed around the lung the area of emphasis was integrated to give the number of counts of radioactivity contained within it. The frame was next shifted to contain the image of the left lung, placing the medial border immediately adjacent to where it had been for the right lung. The area of the left lung was integrated for the number of counts. The area of interest

Fig. 7 Radiograph of rabbit chest showing radiation portal. (Magnif. x1.2)

was always the same size for each pair of lungs to offset to some extent the influence of background radioactivity. Due to the limitations of the computer, corrections could not simultaneously be made for the background radioactivity and the aberrations of the system. Background was felt to contribute negligibly to the total count in each frame. (It can be shown mathematically that the background radiation has the effect of offsetting any deviation from the equal distribution of the isotopes between the two lungs. The observed changes are thus smaller in magnitude than the actual changes. /Appendix 5./) Good separation of the image of the two lungs was obtained by orientation of the antero-posterior diameter of the chest in the vertical plane. In the few instances where this had not been achieved initially it became apparent when the animal was positioned under the camera and was corrected before the image was recorded. Care was taken to place the animal exactly at the center of the screen to minimize the effects of uneven collimation response and distortion. Each computer image was recorded using 50 μCi of ^{131}I-MAA and an "exposure time" of 180 seconds. The scintiphotos were made with 15000 counts on each (except in rare instances.) The camera was set for an average energy of 360 kV with a 20% window for the ^{131}I.

At irregular intervals several different estimations of distribution would be made from one frame and the results compared for internal consistency. (The variation was rarely more than a few tenths of 1%.)

For the studies of acute changes after 4500-6000 rads 113m In was used, bound to gelatin aggregates, because the short half life permitted rapidly repeated studies. During this study the computer was used to subtract the pre-injection image from that following the injection, so

that each result is corrected for the previously given activity and shows the flow at the time of the most recent injection only.

All the above rabbits were studied twice prior to irradiation to determine the base rate of blood flow to the right lung. Following irradiation they were studied at frequent intervals during the first 4 to 6 weeks and then less often.

The rabbits which were irradiated in order to obtain histologic sections were given the usual two studies to determine base line flow rates and then were not studied again after irradiation until just prior to sacrifice. They were studied in the usual manner with ^{131}I-MAA. Immediately following the study during which no ether was used, the rabbit was sacrificed by giving an overdose of pentobarbital. The thorax was opened, lungs, heart and mediastinum were removed en bloc, the hili were ligated and transected proximal to the ligature. Mediastinal tissue was removed. The lungs were studied again separately under a radiation detector for 3 minutes. (A well counter would have been more appropriate for this task, being less dependent on the geometry of the tissue under the detector, but the isotope doses used overloaded the input of the counter, leading to inaccurate readings.) The lungs were then weighed and immediately placed in formalin and labeled. After at least 3-5 days in formalin a slice of lung tissue was removed from the mid-portion of each lung, including in it both hilar and peripheral structures. The plane of the slice was perpendicular to the cephalocaudal axis of the animal. These slices were sent for histologic section and staining. (The microscopic findings were reviewed with Drs. W. Smith and A. Pellegrini of the Department of Pathology.)

The results were calculated as follows: Flow to the right lung was expressed as percent of total. The baseline flow rate to the right lung for each animal was calculated using the pre-irradiation determinations. This rate was set as 100% for each animal. Subsequent flows were expressed as percent above or below baseline (=100%) so that all changes could be expressed in comparable terms. The standard deviation is calculated from the deviation from the mean baseline flow (100%) in all preirradiation determinations including all values for the unirradiated controls. (Due to malfunctioning of the recording equipment only one recording was usable for some animals. These have not been included in determining the standard deviation.) In all 53 animals were studied 134 times prior to irradiation. This includes 5 control animals studied over a 3-4 month period at frequent intervals.

The statistical test employed was the t-test, comparing the overall mean (100%) to the mean of each experimental grouping (1-day or 5 day interval.) This test combines the standard errors of both means to determine the limits of confidence. In cases where only one point is compared to the overall mean significance is determined by using the standard deviation for the overall group and the usual limits of confidence (95%=$\pm$1.96 S.D., 99%=$\pm$2.58 S.D., 99.9%=$\pm$3.29 S.D.) The correlation coefficient in the studies on excised lungs is the Pearson product-moment correlation coefficient.

Results

The blood flow studies show an increase in blood flow to the irradiated lung in the immediate post irradiation period in the rabbits receiving 1500r and 3000 or 3500 rads. The peak of increased flow occurred on the 8th day and reached statistically significant levels.

Following this a decrease in flow to the irradiated lung occurs in some animals, beginning on about the 20th day. Other animals do not show this decrease. The group receiving 750r shows no early increase but shows a decrease on about the 30th day. (Blood flow results are summarized in Fig. 8.)

There were no gross changes in the lungs of the irradiated rabbits until 30 days post irradiation when the irradiated lung appeared paler than the control, occasionally showing small petechial hemorrhages. In examining grossly the lungs of animals surviving 30 and 60 days it became evident that the posterior, inferior portion of the irradiated lung did not show the pallor noted in the rest of the lung. A sharp horizontal line of demarcation between affected and unaffected tissue was readily visible and it appears that this section fell outside the field of irradiation. Comparison with the scintigrams (Fig. 9 and 10) obtained on other animals shows that in the irradiated animal only the inferior portion of the right lung accumulates the isotope in the last scintigram. (In one animal a small portion of the left lung lying near the midline posteriorly was grossly involved with the radiation changes described above. This animal was the only one in which histologic examination showed involvement of any portion of the control lung.)

The histologic studies show a progressive inflammatory response in reaction to radiation injury. The intensity of the response seems to be dose dependent. The reaction is similar to that described by Engelstad (1934) with a shorter latency period but differing mainly in the absence of clearcut changes in lymph nodes. No fibrosis was seen in the 60 day study period, even in those animals who showed a

Fig. 8 Summary: showing blood flow changes following irradiation with different dosages. Points plotted are interval means from Figs. 1-4
(All regression lines are estimates.)

definite decrease in blood flow.

The studies on excised lungs show that there is good agreement between the results obtained by this method and the methods employed on the study. There is also good agreement with the methods used by other investigators which were employed in the study of the excised lungs.

Blood Flow Studies

The mean and range of blood flow to the right lung for 5 rabbits in the control group studied over a period ranging from 93 to 134 days is shown in Fig. 1 and Table 1. The group also contains 3 rabbits sacrificed at intervals for histologic sections having had only one study after the baseline was determined. The mean flow is within 1 S.D. of the overall mean for the entire study period with the exception of the 31-35 day period , the 55-60 day period and the 60-65 day period, all single determinations. This deviation cannot be explained with certainty and has to be tentatively written off to respiratory infection which affected a number of animals at different times during the study. There were no spontaneous deaths in this group during the study. The conclusion drawn from the control group is that no significant deviations in blood flow from the baseline are observed in the absence of radiation and that no significant deviations are introduced by the method of study.

The mean and range of blood flow to the right lung for 5 rabbits who received 750 rads to the right lung is shown in Fig. 2 and Table 2. The rabbits were studied overperiods from 106 to 141 days. An

TABLE 1 Control (S.D. = 6.0)

Rabbit No.	Sex	Weight (g)	R lung Baseline (% of total)	Control studies (% of baseline)		Post Irradiation Studies (% of baseline) ( ) = days post irradiation									
7	M	3750	49.0	99	101	97(19)	94(18)	86(28)	91(38)	97(43)	94(51)	98(117)			
10	F	4000	46.8	107	93	87(9)	106(14)	100(23)	79(30)	120(37)	115(46)	106(117)			
11	F	4300	48.1	95	105	105(3)	99(10)	94(17)	97(25)	93(34)	99(41)	98(47)	107(54)	107(63)	104(134)
33	M	2925	47.6	98	102	--(12)	109(18)	108(26)	101(93)						
34	M	2700	52.3	100	100	--(12)	90(14)	103(26)	97(93)						
Animals for Tissue Study															
48	M	3025	46.0	(100)	--	134(8)									
65	M	2625	53.8	--	(100)	102(30)									
56	M	2825	44.7	97	103	117(60)									

TABLE 2 750 rads (S.D. = 6.0)

Rabbit No.	Sex	Weight (g)	R lung (% of total)	Control studies (% of baseline)											
4	F	4650	55.8	(100)	--	89(17)	83(27)	84(34)	84(47)	83(61)	94(67)	81(75)	82(141)		
13	F	4400	49.0	93	107	92(3)	87(10)	96(13)	96(25)	79(34)	95(41)	86(47)	102(54)	95(63)	96(134)
16	M	2200	50.6	98	102	05(4)	91(10)	96(24)	101(43)	98(51)	93(107)				
18	M	2325	45.5	102	98	01(3)	95(8)	--(22)	96(35)	119(41)	99(49)	99(106)			
Animals for Tissue Study															
61	M	2600	49.1	97	103	118(8)									
68	M	2800	55.2	105	95	98(30)									
52	M	2725	46.3	(100)	--	96(60)									

Fig. 1 Control - no irradiation. Mean and range for data grouped in 5 day intervals. No. of rabbit and relative position in range shown for each interval.

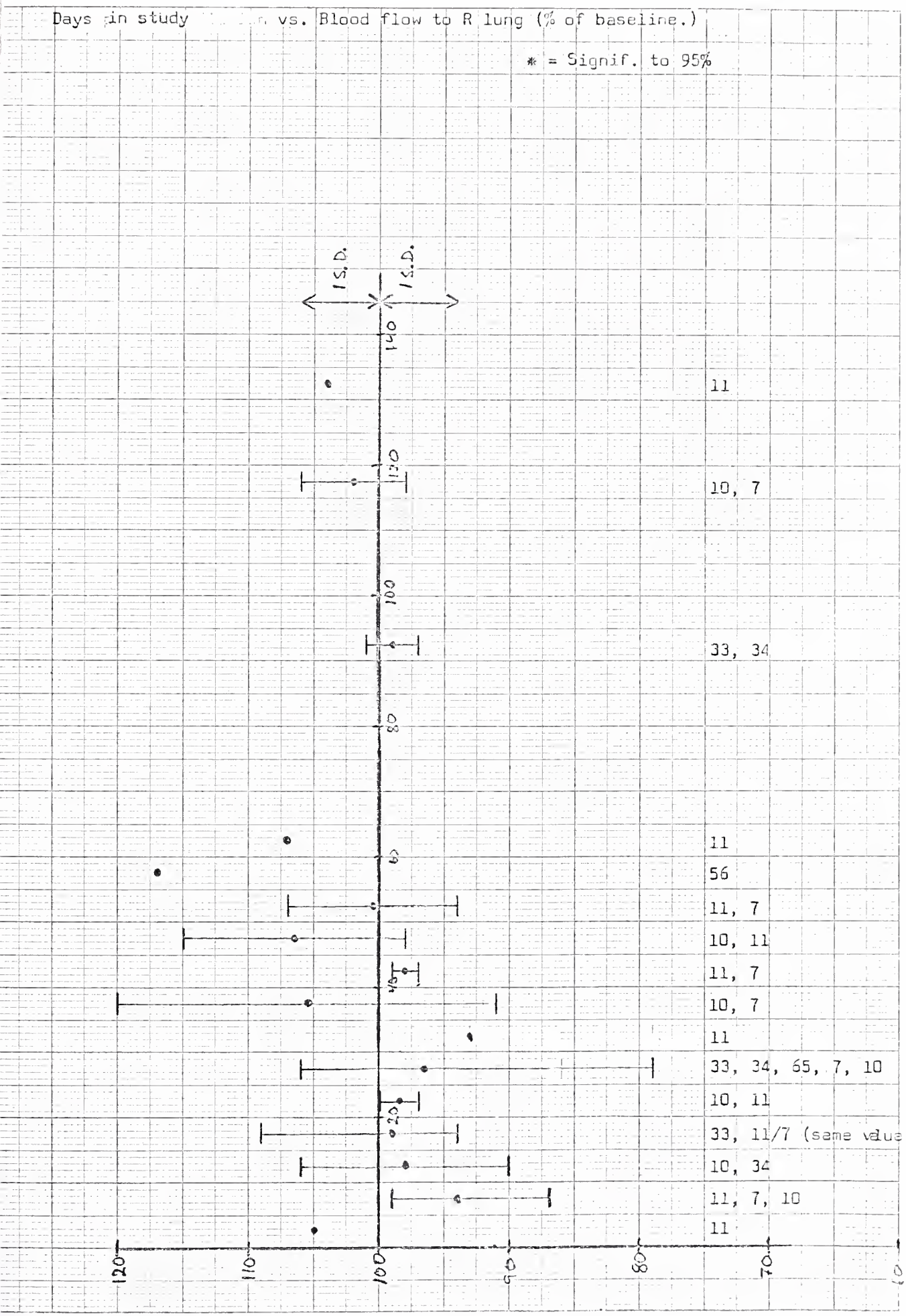
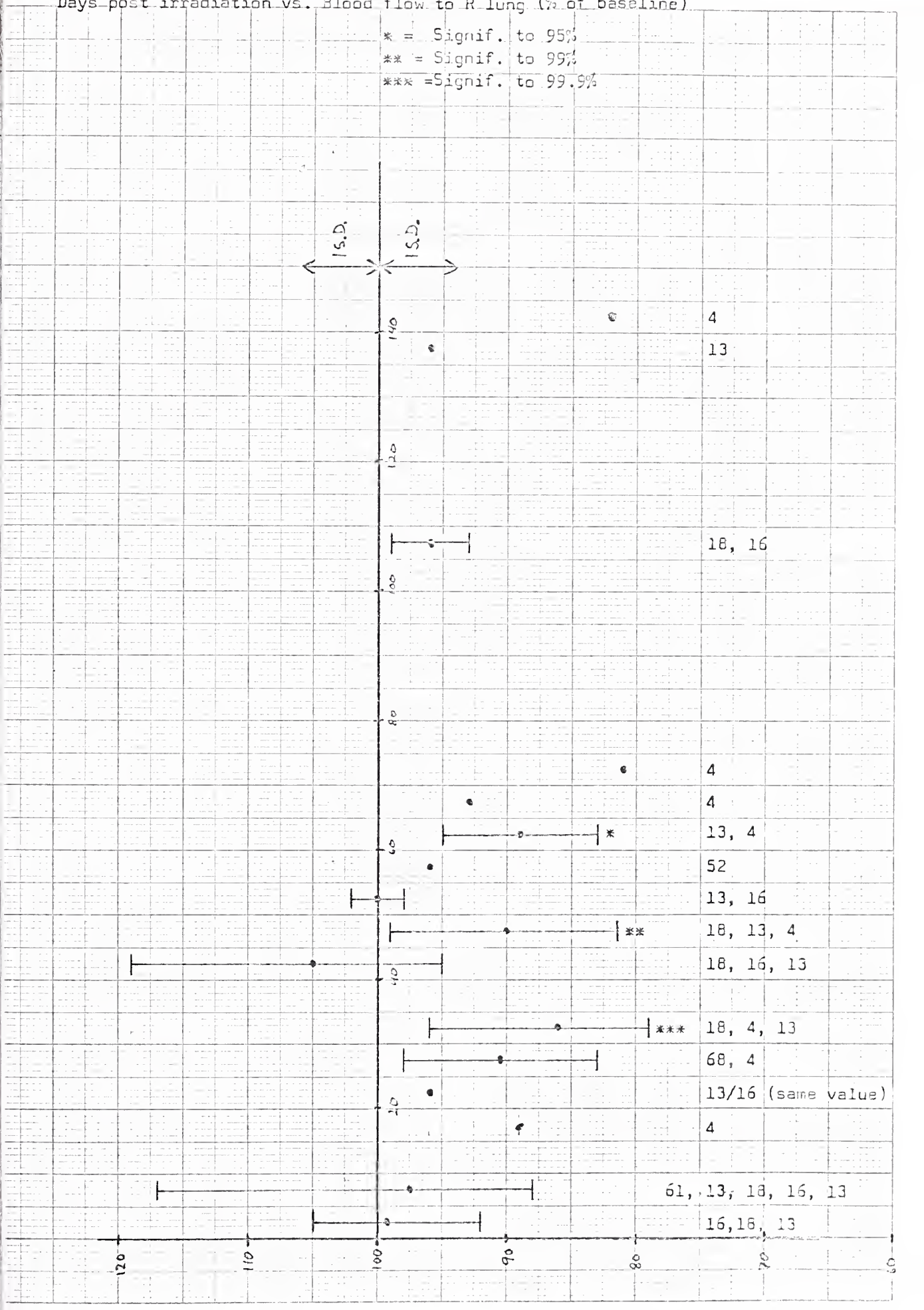


Fig. 2 750 rads. Mean and range for data grouped in 5 day intervals.
No. of rabbit and relative position in range shown for each interval.
Days post irradiation vs. Blood flow to R lung (% of baseline)



additional 3 rabbits were sacrificed at intervals for histologic sections. There is a general trend toward a diminished blood flow, beginning at the time of radiation and reaching statistical significance in the 31-35 day period (99.9% confidence) and in 4 of the 9 subsequent 5-day periods in which determinations were made. (The 6-10 day period would have shown a significant decrease /95% conf./ except for the inclusion in the group of rabbit 61 which showed a marked increase in blood flow, similar to that seen in groups receiving higher doses of radiation /see below/.) There was no depilation observed. There were no spontaneous deaths during the study. The conclusion drawn from this group is that rabbits tolerate well a single dose of 750 rads to one hemithorax which may rarely result in an increased flow in the acute stages but the typical pattern is one of marginally diminished blood flow over time, reaching statistically significant levels 1 month after irradiation and continuing slightly diminished to the end of the study at 4-1/2 months.

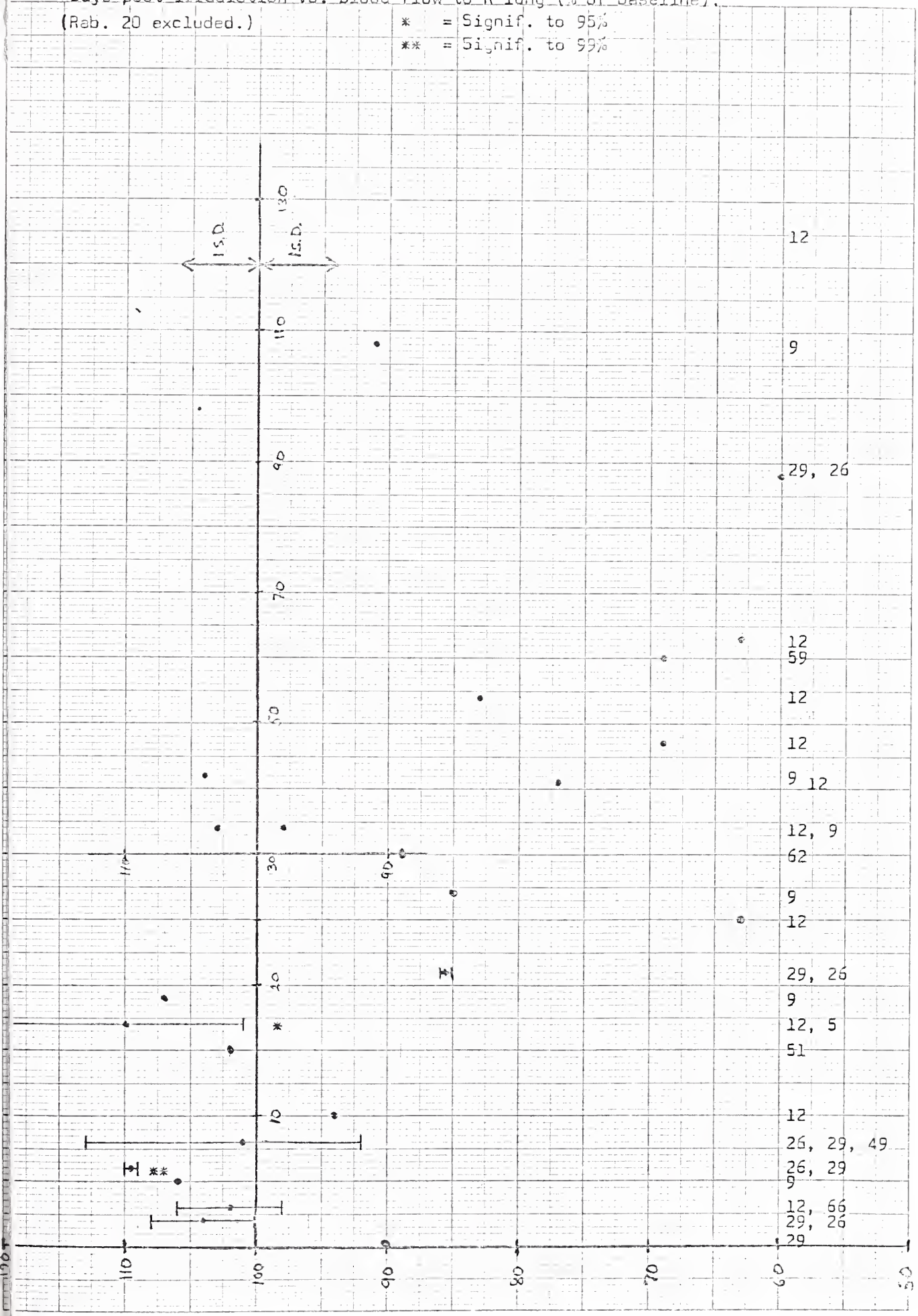
The group receiving 1500 rads is shown in Fig. 3 and Table 3. The group includes 6 rabbits studied serially and 5 rabbits studied only once at varying durations of survival and then sacrificed for tissue sections. Thereis an early increase in blood flow to the right lung, reaching 110% on the 6th day post irradiation. This represents a statistically significant increase (95% confidence.) Another statistically significant increase is noted on the 17th day. The statistical significance derives mainly from rabbit no. 12 which showed great variability throughout (see Tabel 3.) This great variation makes themeaning of this observation somewhat uncertain. Significantly, decreased blood flow occurs first on the 21st day post irradiation. The

TABLE 3 1500 rads (S.D. = 6.0)

Rabbit No.	Sex	Weight (g)	R lung Base line (% of total)	Control studies (% of baseline)			Post Irradiation Studies (% of baseline) ( ) = days post irradiation									
5	F	3860	47.6	--	(100)		101(17)									
9	M	4275	52.0	99	108	93	106(5)	—(14)	107(19)	85(27)	98(34)	104(42)	91(108)			
12	F	3625	45.5	(100)	--		106(3)	94(10)	119(17)	63(25)	103(34)	77(41)	69(47)	83(54)	63(63)	49(124)
20	M	2275	52.5	97	103		96(2)	93(8)	89(15)							
26	M	2600	48.4	88	109	103	--(0)	100(2)	110(6)	113(8)	85(21)	46(88)				
29	M	3175	46.2	99	91	110	90(0)	108(2)	109(6)	99(8)	86(21)	60(88)				
Animals for Tissue Study																
66	M	2375	55.9	99	101		98(3)									
49	M	2725	51.1	96	104		92(8)									
51	M	2500	53.0	105	95		102(15)									
62	M	2825	53.6	104	96		84(30)									
59	M	2575	47.2	102	98		69(60)									

Fig. 3 1500 rad Mean and range for data grouped in 1 day intervals.
No. of rabbit and relative position in range shown for each interval.
Days post irradiation vs. Blood flow to R lung (% of baseline).
(Rab. 20 excluded.)

* = Signif. to 95%
** = Signif. to 99%



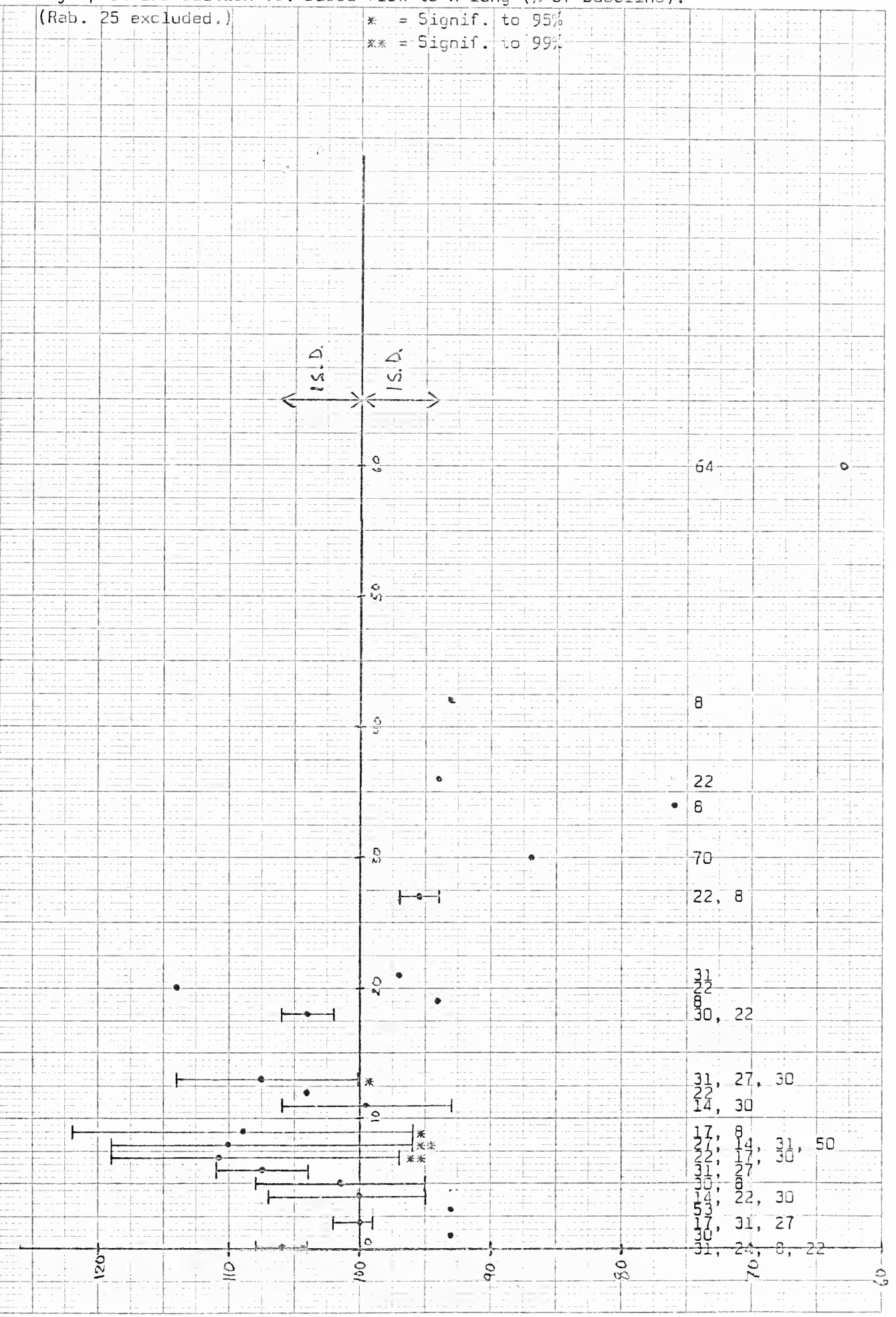
second part of the graph, using a different time scale, shows a marked reduction in blood flow to occur, reaching a level of 50% of base line values on about the 100th day post irradiation. One rabbit, no. 9, does not follow this pattern and shows a flow of 91% on the 108th day. Rabbit 12 was noted on the 43rd day to have depilation over the right hemithorax, progressing to a totally denuded area involving the front, side and back with sharply delineated borders corresponding very closely to the field of irradiation. Rabbit 59 was observed to have similar depilation and dry desquamation, well under way on the 60th day at the time of sacrifice. One rabbit, no. 20, died spontaneously on the 16th day, showing a progressive decrease in flow to the right lung beginning from the first study after irradiation. (Histologically, both lungs of this animal showed very heavy inflammatory infiltrates, consistent with a bacterial pneumonia.) Another rabbit died from a previously tolerated dose of anesthetic on the 17th day, apparently showing increased reaction to the drug. Thus, in this group there is a significant early increase in blood flow to the irradiated lung, followed by a marked reduction in some animals and no significant deviation from base line in others. There is marked variability, even within the same animals, possibly resulting from intercurrent infection. The dose delivered was lethal in one case and led to altered susceptibility to drugs or depilation in others.

The groups receiving 3000 rads and 3500 rads were combined for purposes of analysis. There was no apparent difference in response between the two groups and because of the high mortaility the groups considered separately did not contain enough animals for statistical analysis. The combined group contained 11 animals for serial study and

TABLE 4 3000 - 3500 rads (S.D. = 6.0)

Rabbit No.	Sex	Weight (g)	R Lung Base line (% of total)	Control studies (% of baseline)			Post Irradiation Studies (% of baseline) ( ) = days post irradiation										
3000 rads																	
8	M	4075	49.9	101	102	97	105(0)	95(3)	96(9)	--(14)	94(19)	94(27)	76(34)	93(42)			
14	M	2425	47.9	102	98		107(4)	113(8)	106(11)								
17	M	2025	48.3	102	99		102(2)	116(7)	122(9)								
27	M	2575	45.4	106	94	100	--(0)	99(2)	104(6)	119(8)	109(13)						
30	M	2500	51.2	102	98		93(1)	95(4)	108(5)	97(7)	93(11)	100(18)	106(18)				
3500 rads																	
22	M	2275	44.4	100	91	109	104(0)	98(4)	119(7)	104(12)	102(18)	114(20)	97(27)	94(36)			
24	M	2475	44.9	---	98	102	107(0)										
25	M	2325	52.5	98	102	--	18(0)	95(2)	78(8)	91(13)	90(16)	--(23)	88(26)	---(29)	69(34)	54(41)	12(48)
31	M	2825	47.1	109	94	98	108(0)	100(2)	111(6)	112(8)	114(13)	97(21)					
Animals for tissue Study (3000 rads)																	
53	M	2600	53.7	98	102		93(3)										
50	M	3000	53.0	91	109		96(8)										
69	M	2725	44.7	96	104		--(15)										
70	M	2525	54.4	98	102		87(30)										
64	M	2225	45.3	96	103		63(60)										

Fig. 4 3000-3500 rad. Mean and range for data grouped in 1 day intervals
No. of rabbit and relative position in range shown for each interval.
Days post irradiation vs. Blood flow to R lung (% of baseline).

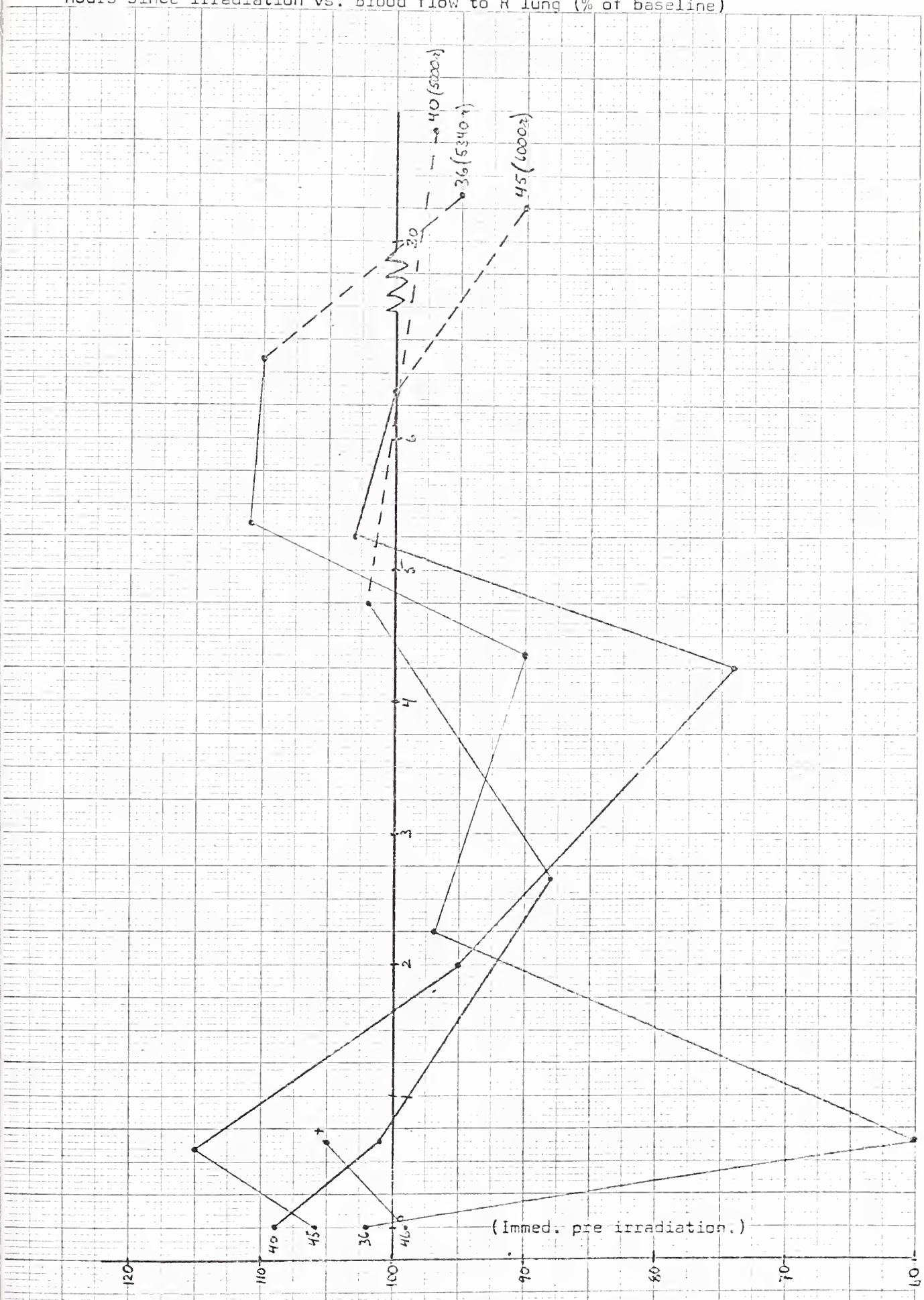


5 animals for single study and histologic examination. (Fig. 4 and Table 4.) One animal died spontaneously on the day of irradiation; 2 received apparent anesthetic overdoses, one on the day of irradiation and one on the 6th day. The remainder were studied over a period from 14 to 98 days. One of these, rabbit 25, showed a very atypical response having a flow to the right lung of only 18% on the day of irradiation (following cardiorespiratory arrest and resuscitation.) Subsequently, it remained below baseline for the entire study. Because the possibility of a pulmonary embolus was raised this animal was excluded from the summary in Fig. 4. The remaining 7 rabbits are shown in Fig. 4. The rabbits which were studied on the day of irradiation showed an increase in blood flow which was "almost significant" to 95% ($\underline{t}$ = 1.96 with a critical value of 1.98 or greater required for significance.) A highly significant (99% confidence) increase in blood flow is noted on the 7th and 8th day, reaching 111% and 110% respectively. Significant increases (95% confidence) were noted on the 9th, 13th and 20th days. (The 20th day is a single determination, however.) The inclusion of rabbit 25 would have eliminated the significance of the increases on the 8th and 13th days. The mortality in this group was high. Two rabbits died spontaneously on the 14th day, one (from the tissue section group) on the 15th day, one on the 20th day, and one on the 21st day after having shown clinical evidence (nasal discharge) of a respiratory tract infection 5 days previously. The remainder showed signs of depilation developing 3-5 weeks after irradiation. Rabbit 25, the longest survivor - 150 days at sacrifice - had large ulcerated areas corresponding to the area of irradiation, contractures limiting the movement of the right forelimb. Autopsied rabbits showed signs of intraperitoneal bleeding in some and bleeding from the mouth and nose

TABLE 5 Acute Changes

Rabbit No.	Sex	Weight (g)	Dose(r)	R lung Base line (% of total)	Control studies (% of baseline)		Post Irradiation Studies (% of baseline) ( ) = hours since irradiation						
46	M	2725	4500	52.3	96	103	99(pre)	105(0:40)					
40	M	2550	5000	50.4	99	101	109(pre)	101(0:40)	88(2:40)	102(4:45)	--(5:50)	--(6:50)	97(30:50)
36	M	--	5340	49.5	92	108	102(pre)	59(0:40)	97(2:15)	90(4:20)	111(5:20)	110(6:35)	95(30:20)
45	M	2625	6000	50.9	100	100	106(pre)	115(0:35)	95(2:00)	74(4:15)	103(5:15)	100(6:20)	90(30:15)

Fig. 5 Acute changes after 4500-6000rad.
No. of rabbit and dosage indicated on graph.
Hours since irradiation vs. Blood flow to R lung (% of baseline)



in others indicating injuries to the upper GI tract, some of which was included in the field. (Cf. Engelstad 1934 who notes"ulcus perforans" as a frequent cause of death in those animals who died spontaneously.)

Four rabbits received single radiation doses from 4500 rads to 6000 rads and were studied intensively during the first day. (Fig. 5, Table 5.) The study fails to support the impression from the 3000-3500 rad group that there is an early increase in the blood flow during the 1st 30 hours. There is instead a suggestion of a decreased flow, 3-4 hours, after irradiation which is similar to the observation by Kallman. Since a different isotope (^{113m}In) with a different vehicle (gelatin) and a different technique were employed the confidence limits used in the rest of the study cannot meaningfully be applied here. The finding is present in all the animals, however. The 30 hour study was done using conventional technique and shows no significant changes from baseline.

Studies on Excised Lungs

The result of studies on excised lungs are shown in Table 6. The values obtained from the standard blood flow determinations, based on the same injection of tracer, are shown for comparison. The table shows the counts measured by the radiation detector (corrected for background radiation) for a 3 minute period of counting the excised lungs. The counts in the right lung are expressed as a percentage of the total count. When this is compared to the similar determination on the gamma camera with the lungs in situ we find a correlation coefficient of $r = 0.87$ ($p. < 0.001$).

The data are also expressed in the form of counts per 3 min. per

Table 6. Studies on Excised Lungs

Group	Rab. No.	Lung	Weight	Cts/3min	R lung % of tot	3min Cts/g	(R/L)	Gamma Camera R lung % of tot.	(R/L)
Ctrl	48	R	6.937	19916	66.6	2870	1.51	61.7	1.61
	(8 d.)	L	5.202	9900		1900			
	65	R	7.874	46687	54.2	5930	0.95	54.7	1.21
	(30d.)	L	6.271	39574		6310			
	56	R	6.086	46071	53.8	7680	0.84	52.2	1.09
	(60d.)	L	4.323	39567		9140			
750 r	61	R	6.436	42507	59.5	6610	1.04	57.8	1.37
	(8 d.)	L	4.549	28927		6350			
	68	R	7.174	26631	59.7	3715	0.95	54.1	1.18
	(30d.)	L	4.602	18082		3925			
	52	R	6.051	32756	47.2	5410	0.71	44.4	0.78
	(60d.)	L	4.591	35155		7650			
1500 r	66	R	6.658	77252	59.4	11600	0.88	54.9	1.22
	(3 d.)	L	4.053	53353		13150			
	49	R	6.817	24494	53.0	3590	0.83	46.8	0.83
	(8 d.)	L	4.978	21697		4350			
	51	R	4.991	24568	53.8	4930	0.79	54.1	1.18
	(15d.)	L	3.381	21081		6240			
	62	R	7.787	39337	50.5	5060	0.63	44.8	0.81
	(30d.)	L	4.828	38591		8000			
	59	R	6.850	32375	42.9	4730	0.60	32.7	0.49
	(60d.)	L	5.494	43110		7850			
3000 r	53	R	6.275	26976	59.0	4300	0.83	50.0	1.00
	(3 d.)	L	3.622	18716		5170			
	50	R	6.203	32220	65.8	5190	1.06	50.8	1.03
	(8 d.)	L	5.004	24437		4890			
	69	R	(Died prior to study)						
	(15d.)	L							
	70	R	6.791	48508	53.4	7150	0.79	47.5	0.90
	(30d.)	L	4.682	42183		9020			
	64	R	7.336	34467	39.5	4700	0.49	28.6	0.40
	(60d.)	L	5.509	52749		9570			

gram of lung tissue. The ratio of counts per gram of the right to the left lung has been calculated. This is similar to the statistic used by Johnson et al. (1970) and differs only in using counts per 3 min. instead of 1 minute. The data from the gamma camera studies have been rearranged to show the same ratio. Comparing the ratios obtained by these two methods we find a correlation coefficient of $r = 0.88$ ($p. \quad 0.001$).

The method thus shows good internal consistency for different methods of determining the distribution of the injected isotope. (The consistency of different determinations using the same method, both for the same and different injections of isotope have been shown above.) It also shows that the results can be meaningfully compared to those of other investigators employing different ways of calculating and displaying the data.

Tissue Studies

In general, the findings are similar to those of Engelstad (1934). The intensity of the reaction described below varied with the intensity of the dose. Signs of radiation injury appeared in a progressive fashion in the irradiated lung. The first change to appear was pulmonary oedema, seen in the shortest survivors (3 d. and 8 d.) This was followed by an acute inflammatory infiltrate with polymorphonuclear leukocytes (poly's) in the arterial walls and interstitial tissue, leading to an increased cellularity. The quantity of this increased cellularity remained approximately constant but with a change in the character of the cell population. The acute inflammatory cells were replaced by chronic inflammatory cells around the 2nd week. Proliferation

and shedding of alveolar cells began about 1 week later. At one month perivascular and peribronchial proliferation was seen with some minimal fibroblastic activity. Macrophages appeared at this time, sometimes in great number. At two months, fat-laden macrophages were numerous and islands of bone formation were seen in the higher dose animals. No fibrosis was seen at two months.

Damage to the lung structures varied in some cases from the inflammatory response. No pleural reactions were noted in any of the animals, which was also the case in those of Engelstad's animals which received "subepidermicidal" doses. The bronchial epithelium showed increased secretion at 3-30 days in some animals. (The presence or absence of secretion could not be correlated to the dose.) Shedding of epithelial cells occurred at one month and later. Loss of cilia with metaplasia was seen at two months. Alveolar cells were seen shed into the alveoli and bronchi at 2 months. Vascular changes consisted mainly of congestion of the vessels in the early stages. Minimal endothelial damage and some perivascular infiltration around the medium-sized arterioles were noted at one and two months. Most vessels showed no change. Thrombi were rarely seen (and there was some question as to their pathologic significance). One animal which showed extensive amounts of what appeared to be thrombus was no. 50, sacrificed at 8 days following 3000 rads. This animal failed to show the expected increase in perfusion of the irradiated lung which should have been at its peak at the time of sacrifice. The thrombus could possibly explain this and in general shed some light on the great amount of variability in the high dosage groups.

Changes in the lymph nodes are also described by Engelstad and many

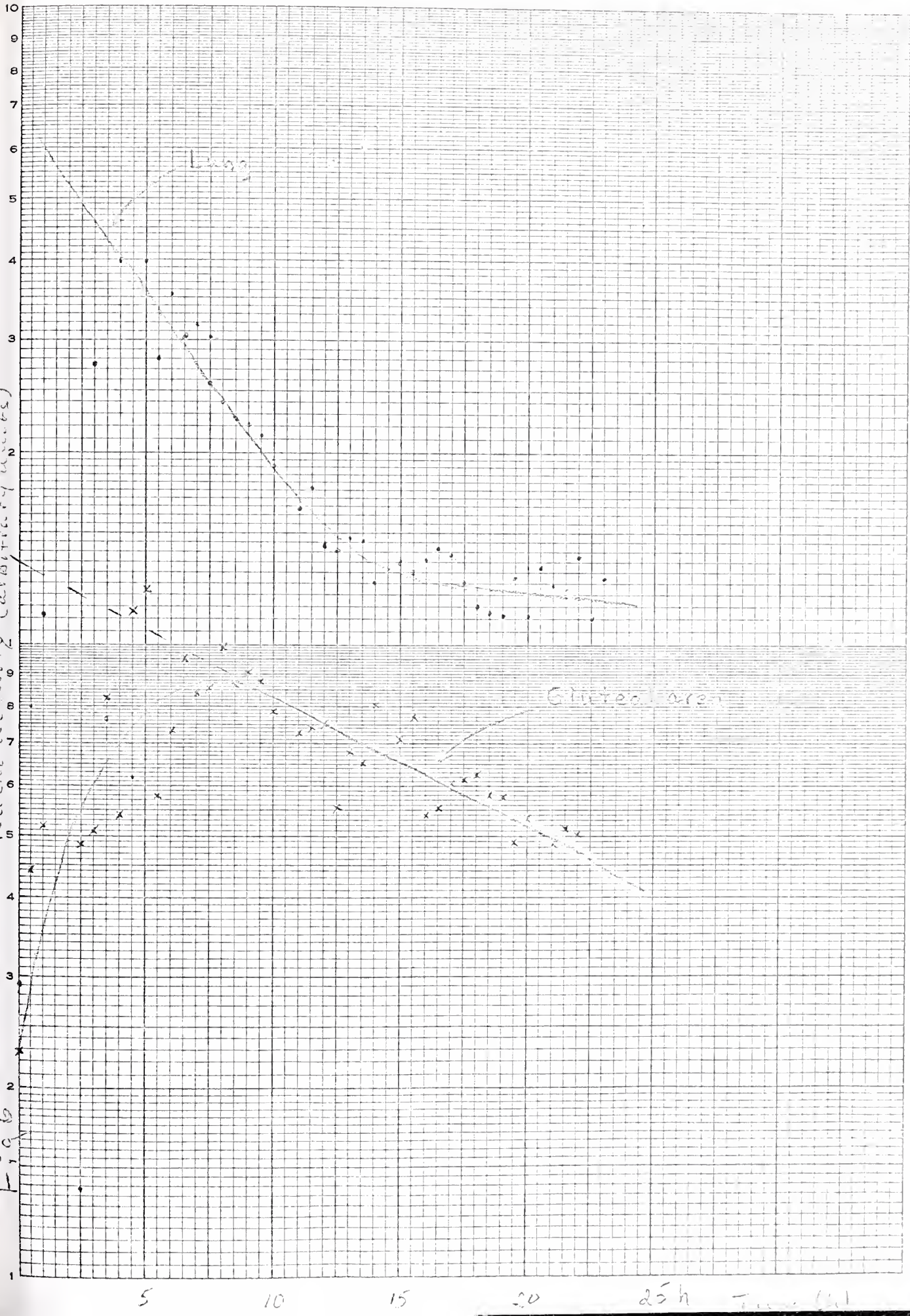
other authors. Engelstad, studying rabbits noted degeneration of the lymph follicles within hours of the radiation with regeneration beginning in the course of the first week and being completed by 2-3 weeks. In the present study the number and size of lymph nodes was smaller in the irradiated lung than in the control at 3 days and 8 days. (The 3000r-8 d. rabbit, no. 50, had large active lymph follicles in both lungs but fewer in the irradiated lung than in the control.) Rabbits 14 and 17 which both died spontaneously 14 days after having received 3000 rads had no lymph tissue on either side in the sections studied, the only animals to show this phenomenon. All the remaining animals showed no appreciable difference in lymph nodes between irradiated and control lungs.

Thus, it appears that the histologic changes are largely in agreement with the classic descriptions of radiation damage, however, with a slightly less than clearcut sequence of changes in the lymph nodes.

Clearance of MAA from the Lung

One rabbit was studied to determine the rate of disappearance of MAA from the lung. The injected rabbit remained in front of the camera for a 24 hour period during which a picture was taken every 30 minutes. The results are shown in Fig. 6 and bear out the reports of similar studies in the literature. (Wagner and Tow, 1967) The half life of the isotope in the lungs is approximately 6 hours. Fig. 6 shows the simultaneous disappearance from the lungs and appearance of the label in the body with a slowly decreasing amount as the isotope is cleared by the kidneys. The reference area in the body was an area over the gluteus muscles, away from the kidneys and bladder. The leveling

Fig. 6 Clearance of ^{131}I-MAA from rabbit lung, also showing the appearance of label in a neutral body area (gluteal musculature.)



off of the curve showing disappearance from the lung is probably due to the inclusion of the heart in the field of study. The blood pool in the heart contains a considerable amount of isotope after the label has been cleared from the lungs.

Discussion

The increased blood flow following irradiation demonstrated in the present study has not previously been reported. The increase is almost certainly entirely pulmonary arterial flow since the bronchial artery receives blood only after its passage through the lung which filters out the MAA particles. Further, the increased trapping of particles in the irradiated lung indicates that the increased flow occurs on a capillary level since larger arterio-venous shunts would not trap the tracer particles but let them escape into the systemic circulation. The papers of Teates (1968) and Johnson et al. (1970) both give some evidence for such an early rise which, however, does not reach any kind of statistical significance and is not further commented on. Their main interest is in the reduction in blood flow occurring over long periods of time, shown also in this study. The blood flow studies bear out the reports of those who suggest on histologic grounds that the earliest reactions to radiation occur in the vascular system, in that the earliest changes are here.

The value of blood flow changes as a prognosticator for future damage was one of the questions asked at the outset. Examination of Tabel 1 shows that in the Control group there was essentially no deviation from baseline although large individual variation could result in presumably fortuitous statistical significance in small groups. (On the other hand the significance may not be fortuitous

but may reflect the influence of factors other than radiation, such as infection.) The animals in Table 2 show no early increase in blood flow and minimal or no subsequent reduction, with survival not being affected over the period of the study.

In Tabel 3 the results are anything but clearcut. Rabbit 9 shows a slightly elevated blood flow early but goes on to show no significant decrease. Rabbit 12 shows a very slight early increase but goes on to manifest significantly decreased flow after a very erratic course. Rabbit 20 shows no increase but a steady decline in blood flow until the time of death. Rabbits 26 and 29 show large early changes and progress to show marked reductions in flow. Increased flow here seems to have no predictive value on eventual outcome.

In Table 4 the majority of the animals show early increases in flow. The exceptions are rabbits 8 and 25. Rabbit 25, discussed above, was excluded from the summary in Fig. 4 because of its atypical response following cardiac arrest during the irradiation. The spectacularly long survival of this animal also set it apart from others in this group. Rabbit 8, with the exception of the day of irradiaton never showed flows exceeding the base rate. It was part of the first sending of rabbits and weighed 4 kg versus an average of 2.5 kg for the remainder. Its larger size may have made it less susceptible to the radiation delivered to a standard field because of a decreased depth dose.

It very consistently occurs as the lowest point in the range indicated in Fig. 4. All the animals died spontaneously (except no. 25) presumably due to the effects of radiation. The magnitude of the rise in blood flow had apparently no relation to the duration of survival,

with one animal showing an increase to 122% of baseline dying at 14 days, while another with the same increase was one of the longest survivors.

Thus, the observed increases in blood flow do not seem useful as prognosticators in the present study.

Teates made the unexplained observation that there was an increase in pulmonary diffusing capacity for CO (DLCO) which occurred during the first and third weeks (1965, restated in 1966.) The timing of the increase in DLCO is similar to that observed for increased blood flow in the present study. However, Turino et al. (1969) state that "the rate of pulmonary blood flow should have a negligible effect on the transfer of gas from alveolar air to blood"; i.e., the DLCO should not be affected. Furthermore, Teates found no increase in O_2 consumption and CO_2 production, both of which are "largely related to blood flow." The DLCO is directly related to the total capillary volume of the lung when the diffusion constants of the membrane and red blood cells remain the same. (Roughton and Forster, 1957) Thus an increased DLCO would be indicative of an increased capillary volume in the irradiated lung, this has been seen microscopically as the well described hyperemia which follows irradiation.

The observation of an initial decrease in the blood flow in the first hours following doses of 4000-6000 rads is in agreement with the unpublished data of Kallman. The decrease is greatest for the rabbit receiving the largest dose. It appears to coincide in timing with the initial response of oedema following radiation injury, as described in the literature. No histologic studies were undertaken on rabbits surviving less than 3 days in the present study.

There is further agreement with Kallman's report on the increase in blood flow. The 1500 rad group showed peak blood flow on the 6th day following irradiation, the 3000-3500 rad group showed its peak on the 8th day. In Kallman's study in irradiated mouse sarcoma the increase occurs later with larger doses. It is further of great interest that a very different tissue, mouse sarcoma, behaves in a fashion very similar to that observed for lung tissue in the present experiment. This strengthens the impression that the observed blood flow changes are generalized phenomena occurring in blood vessels exposed to radiation. Thus, the finding of increased pulmonary blood flow in the acute period following irradiation seems to have uncertain importance, either for prognosis (except as a general indicator of heavy doses of irradiation, when present) or in explaining the unexplained findings of others. It may be of some value in influencing schedules for radiation therapy for maximal efficiency. Since tumor camage is greatest in oxygenated cells, the increased perfusion which follows large doses of radiation (which were shown by Kallman et al. to occur in tumors as well) could be taken advantage of by fractionation of the dose so that a relatively larger dosage is delivered at the time of maximum susceptibility. Instead of daily equal doses, an initial large dose could be given followed by another large dose around eight days thereafter when tumor oxygenation should have reached a peak value. This could possibly be combined with oxygen inhalation and change in position.

The finding of increased perfusion following high dose radiation therapy thus stands by itself as an unexplained finding with potentially useful therapeutic importance.

Conclusion

The present study shows acute changes in blood flow to irradiated lung tissue. Early, within hours, there is a slight decrease in perfusion, which, combined with the observation of hyperemia, indicates vascular stasis. This stasis appears similar to that observed as a part of the inflammatory response and may be on the basis of venous constriction or vascular oedema. Following this there is an increase in the blood flow to the irradiated tissue which is dose dependent and peaks on the 8th day. This is not accompanied by any definite change in the histologic picture. Around 1 month after irradiation the blood flow to the irradiated lung decreases, reaching 50% around 100 days after irradiation. These changes call into question the customary fractionation of radiotherapy. The initially decreased flow needs to be determined further to determine if it persists at 24 hours post irradiation. Any such decrease would cause a decreased oxygenation which would protect the tissue from the radiation damage which is the sought after therapeutic effect. On the other hand, the increased flow on the 8th day would increase the tissue oxygenation - leading to increased radiosensitivity. The findings of the present study call for further investigation of radiation induced perfusion changes and suggest a careful re-evaluation of radiaton therapy fractionation schedules.

Summary

Rabbits were irradiated using single doses of 750r, 1500r, and 3000r (or 3500r) to the right lung. In addition, a small group of rabbits were given single doses from 4500r - 6000r. Rabbits from

each group were studied serially for pulmonary perfusion changes. Perfusion was determined by digital quantification of ^{131}I-MAA lung scans recorded with a Pho/Gamma III gamma camera using a pinhole collimator. (For studies of short term changes ^{113m}In bound to gelatin particles were used.) Other rabbits in each group were sacrificed at intervals after irradiation to determine radiation induced histologic changes in the lung, in order to correlate histologic and perfusion changes.

Histologic changes were similar to those described in the literature following radiation injury. Perfusion changes, hitherto undescribed, were observed. Control rabbits showed no changes. Rabbits receiving 750r showed a slight decreased in perfusion of the irradiated lung, reaching significant levels at 30 days. Rabbits receiving 1500r showed an initial increase, reaching a significant peak of 110% of baseline on the 6th day, followed by a decrease beginning on the 20th day reaching 50% of baseline on the 100th day. In the 3000-3500r group a significant increase to 110% of baseline occurred on the 7-9th days. The mortality in the group was very high and very few animals survived for an extended period. In addition, rabbits receiving 4500-6000r were studied intensively during the first hours after irradiation and showed a decrease in pulmonary perfusion at 2-4 hours after irradiation.

The observed radiation induced perfusion changes suggest a re-evaluation of radiation therapy fractionation schedules.

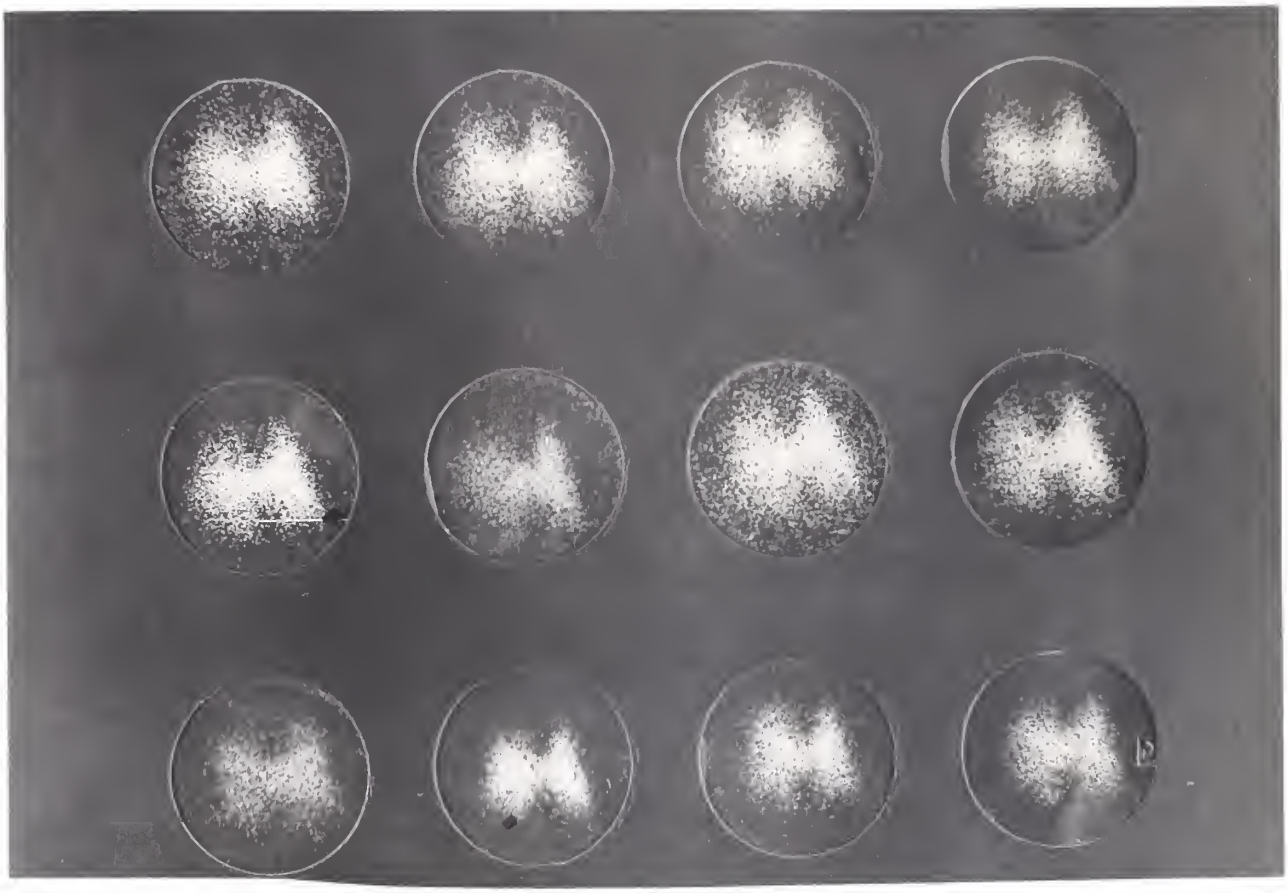


Fig. 9 Sequence of lung scintigraphs from a control rabbit.

(Rabbit 11. Days since assignment to control group.)

pre	pre	3	10
17	25	34	42
48	55	64	135

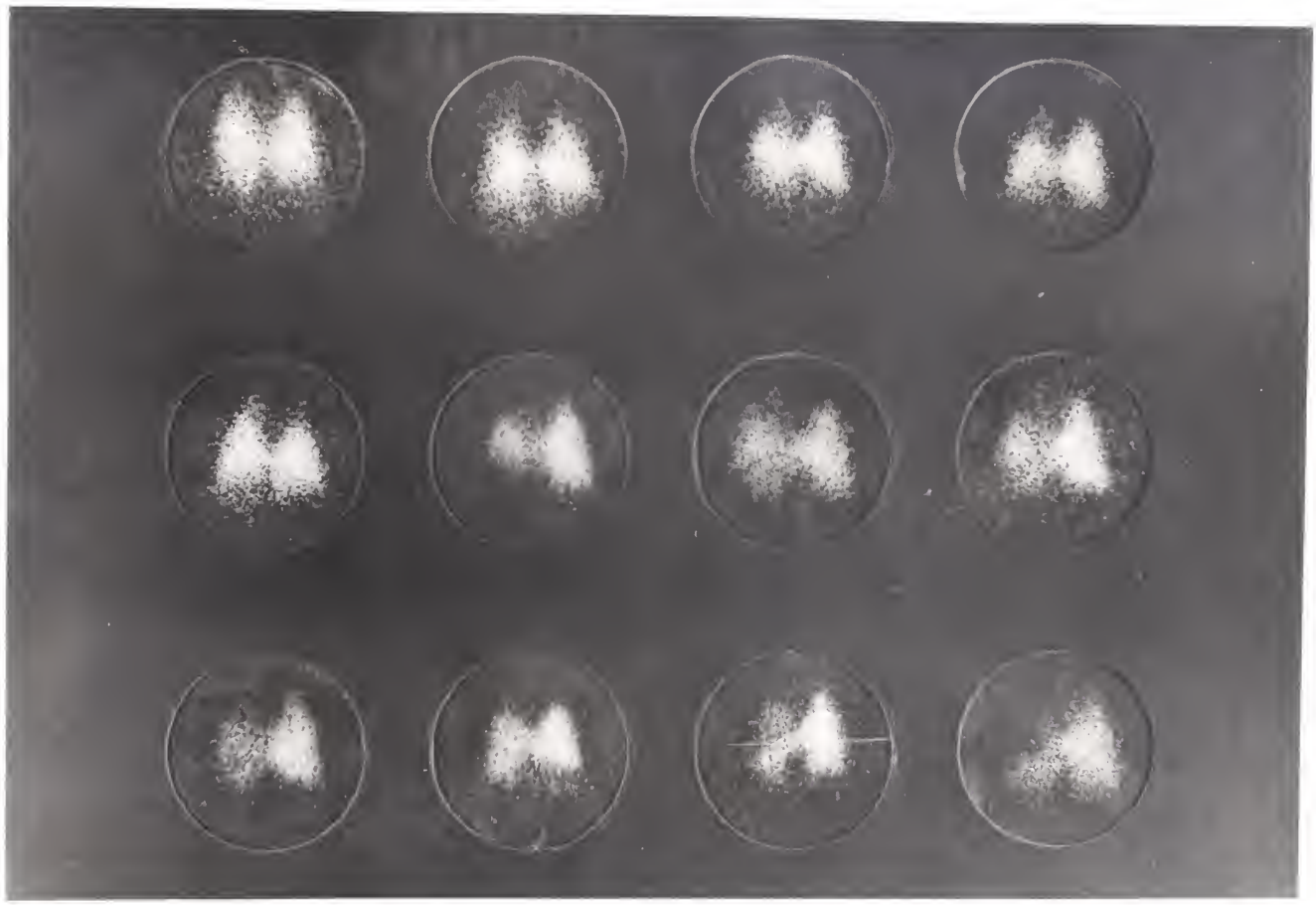


Fig. 10 Sequence of lung scintigraphs from an irradiated rabbit.
(Rabbit 12. 1500 r. Days post irradiation.)

pre	pre	3	10
17	25	34	42
48	55	64	125

Appendix 1.

"Est-ce a dire toutefois que les radiations obscures n'ont exerce aucune influence a sur les animaux en experience? ... Mais que penser de cette sclerose pleurals si etendue, de cette symphyse totale de la plevre inoculee, existant exclusivement chez les animaux soumis, sclerose a rapprocher de celle observee par Destot au niveau de la surface cutanee des cobayes soumis aux radiation ... frappant rapidement toute l'etendue de la surface pleurals, retractant le poumon determinant la surelevation et l'aplatissement du thorax, du diaphragm ... Nous nous contentons pour le momemt de signaler ces faits sans chercher a savoir si nous devons les attribuer exclusivement aux rayons X our s'il convient d'en accorder une part a l'action du champ electrostatique. ... Les resultats pathologiques experimentaux imposent toute reserve et plaident, quant a present, contre l'application du rayons X, au traitement de la tuberculose humaine." Bergonie and Teissier (1898, pp. 351-2)

Appendix 2.

"Die Gefassveranderungen sind in der Regel wenig ausgesprochen. Die Kapillaren haben ihre Kontrationsfähigkeit (Adrenalin, Pituitrin) und ihr Dilatationsvermögen (Histamine) bewahrt. Aus diesem Grunde erscheint es unwahrscheinlich dass die Reaktion einum primärem Gefasschaden zuzuschreiben wäre." Engelstad (1934, p. 88.)

Appendix 3. a), b), c)

a) "/Die ...Lungen wiesen Gewebsreaktionen auf, deren Vielfalt im wesentlichen/ auf eine Permeabilitetsstörung der Lungenkapillaren, -arterien und -venen zurückgeführt werden kann." Bässler and Buchwald (1966, p. 193)

b) "Hyperämie und Ödem der Lunge sind die frühesten and zugleich kennzeichnenden Gefässreaktionen. Von den ersten Stunden nach der Bestrahlung an werden diese Reizformen deutlich und äussern sich in Weitstellung der Kapillaren mit aneurysmatischen Ektasien, ... Bereits nach 2 Tagen beobachteten wir ein perivenöses Ödem mit auflockerung der Wandtextur und Schwellung von Endothelzellen." p. 193-4

c) "Die elektronenmikroskopischen Untersuchunger zur Radiopathologie der Lunge haben Gezeigt, dass die alveolo-kapilläre Membran der Lunge in den ersten 12 Stunden nach einzeitiger Bestrahlung mit 2000 R Veränderungen aufweist, die teilweisse schon nach 4 and 8 Stunden durch eine vermehrte Membranvesikulation des Endothels, durch eine verbreiterung der Basalmembran und der Deckzellen erkennbar sind. Der als Cytopempsis /Moore and Ruska, 1957/ bekannte Transportmechanismus von Flüssigkeit aus dem Blutplasma durch Endothel, Basalmembran önd Alveolardeckzellen bis in die Lungenalveole nimmt im Verlaufe der ersten Tage nach der Bestrahlung rasch zu. Die Veränderungen in Feinstruktur im Cytoplasma der Endothel- und Alveolardeckzellen zeigen, dass die Ödemflüssig-keit transzellulär in die Schichten der alveolo-kapillären Membran gelangt. Im Endothel können Ansammlungen von wässriger Flüssigkeit zu umfangreichen Blasenbildung führen. ... Mit Ausbildung der interstitiellen Entzündung nach der zweiten Woche wird deutlich, dass das Ödem mehr und mehr Eiweiss enthält. ... Das heisst, dass nun neben der quantitativen Zunahme der Gefässpermeabilität auch ein qualitativer Effekt hinzutritt als aus dem Transudat ein eiweissreiches Exsudat das später auch Fibrin enthält." p. 202-3

Appendix 4.

"/Dans les/ 15 premieres secondes qui suivent le placement de l'animal en position orthostatique la masse sanguine pulmonaire suit les lois de la pesanteur et se collecte aux bases. Par contre, lorsque l'injection tracante est faite 20 s., 40s. une mn et plus apres que l'animal a ete place en position verticale, la repartition sanguine devient homogene dans les deux champs pulmonaires et il n'est plus possible d'affirmer une repartition significativement differente de celle que l'on avait observee, l'animal etant en position horizontale." Fernandez et al., 1969, p. 114.

Appendix 5.

Discussion of the influence of background radiation on observed values

Blood flow is measured as the amount of radioactivity in each lung.

Let x= the proportion of radioactivity in the R lung and

y= " " " " " " L "

The total radioactivity in both lungs is $x + y = 1$ (1)

The proportion of the total amount of radioactivity in the R lung can be written:

$$R + \frac{x}{x + y} \quad \text{or, inserting (1):} \quad R = x \tag{2}$$

This is the equation for a straight line with a slope of 1 and an intercept of 9. Let a= the background reaction (which under the conditions of this ecperiment is equal for the R and L lung.) The measured radioactivity then becomes:

$$x' = x + a \quad \text{and} \tag{3}$$

$$y' = y + a \tag{4}$$

The proportion of the total in the R lung becomes:

$$R' = \frac{x'}{x' + y'} \quad \text{or, inserting (3) and (4):} \quad R' = \frac{x + a}{x + y + 2a}$$

or, inserting (1): $R' = \frac{x + a}{1 + 2a}$ or, rearranging:

$$R' = \frac{1}{1 + 2a} \cdot x + \frac{a}{1 + 2a} \qquad (5)$$

which is a straight line with the slope $\frac{1}{1 + 2a}$ and intercept $\frac{a}{1 + 2a}$.

The slope of R', $\frac{1}{1 + 2a}$, is less than the slope of R, 1, for all positive values of a.

Lines (2) and (5) intersect where $R = R'$, at the point $x = 0.5$, i.e. where the distribution of radioactivity between the lungs is equal.

Thus, R' is greater than R for all values of x less than 0.5 and

R' " " " R " " " " x greater than 0.5.

Thus, the influence of the background radiation is to offset and obscure all departures from the equal distribution of radioactivity between the two lungs.

Q. E. D.

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